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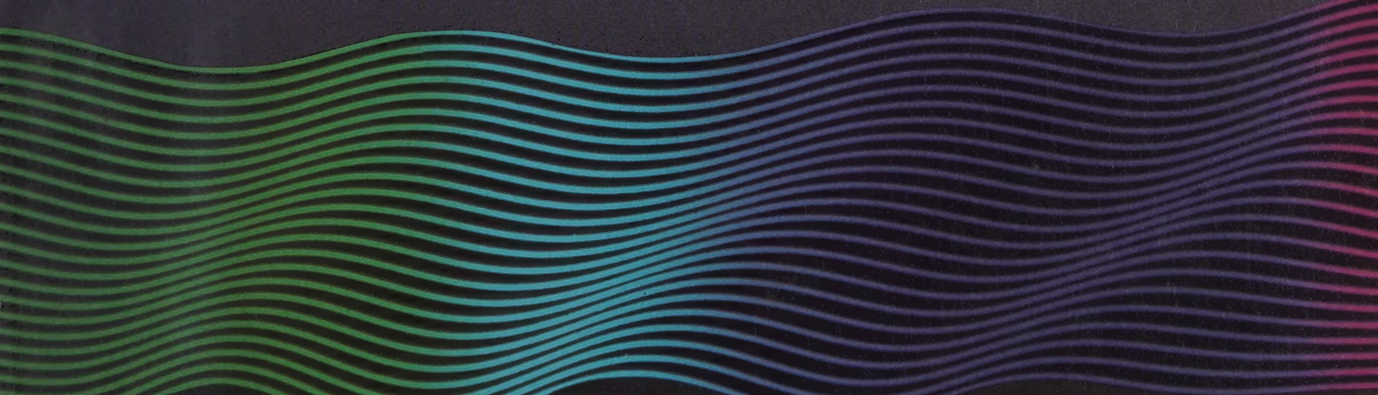

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The Shape of Recruitment Distributions

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The shape of recruitment distributions in marine fish is generally assumed to be lognormal on the basis of empirical evidence and theoretical argument. If recruitment is considered to be an outcome of the probability of survival of individuals or groups of individuals which may contribute to the year class, then it can be shown that the underlying distributional shape will depend on the degree of survival independence and may be nearly symmetrical for bet-hedging species. The underlying distributional shape may be obscured by estimation error, which may suggest a greater degree of positive skewness than actually exists.

On suppose généralement que la courbe de distribution du rendement chez les poissons marins est lognormale d'après l'évidence empirique et des arguments théoriques. Si l'on considère que le recrutement est le résultat de la probabilité de la survie d'individus ou de groupes qui peuvent contribuer à une classe d'âge donnée, alors on peut prouver que la courbe de distribution sous-jacente dépendra du niveau d'indépendance quant à la survie et pourrait être presque symmétrique chez les espèces qui se protègent à cette fin. Le fait que la courbe de distribution sous-jacente peut être embrouillée par l'erreur d'estimation peut indiquer un niveau plus élevé d'asymétrie positive qu'il n'en existe vraiment.

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Following use of the lognormal by Ricker (1958), both empirical evidence (Allen 1973; Hennemuth et al. 1980; Garrod 1983) and theoretical arguments (Walters and Hilborn 1976; Peterman 1978) have been advanced for assuming that recruitment in fish is lognormally distributed. Consequently, it has become common practice to make this assumption in studies involving recruitment (e.g. Peterman 1981; Walters and Ludwig 1981; Archibald et al. 1983; Huang and Walters 1983; Hightower and Grossman 1985; Bergh and Butterworth 1987; Hollowed et al. 1987; Evans and Rice 1988; Armstrong and Shelton 1988; Fogarty et al. 1991). In this paper, some of the empirical evidence and theoretical argument for lognormality are briefly reexamined. An approach is then explored in which individual offspring or groups of closely associated offspring are viewed as having independent probabilities of surviving to recruitment. It is shown that the second, third, and fourth moments of the resulting distribution are sensitive to the degree of survival independence among individuals in the brood. Consequently, it is suggested that life history traits which influence survival independence will alter the distributional shape of recruitment. In bet-hedging species with a high degree of survival independence, the distributional shape may be expected to be nearly symmetrical with a relatively small variance. In this paper, the following sample estimators of skewness (γ_1) and kurtosis (γ_2) are used:

$$\gamma_1 = \frac{n}{(n-1)(n-2)} \sum_{i=1}^{n} \frac{(x_i - \bar{x})^3}{\sigma^3}$$

(1)

$$\gamma_2 = \frac{n(n+1)}{(n-1)(n-2)(n-3)} \sum_{i=1}^{n} \frac{(x_i - \bar{x})^4}{\sigma^4} - 3\frac{(n-1)^2}{(n-2)(n-3)}.$$

Empirical Evidence for Lognormality

In empirical studies of recruitment, lognormality has been used without distinction to describe the distribution of recruitment estimates, the distribution of residuals about a fitted model, and the distribution of values obtained from a ratio such as that of recruits to spawners. Several of these studies, some of which are reviewed below, purport to demonstrate lognormality.

Allen (1973) showed that values of the ratio of returns to escapement for Skeena River sockeye salmon (*Oncorhynchus nerka*) fitted the lognormal distribution reasonably well by taking logarithms and presenting the data as a normal quantile plot. However, a simulation experiment shows that the ratio of two independent random normal variables, x_1 and x_2, with means μ_1 and μ_2 and variances σ_1^2 and σ_2^2, respectively, set at values roughly appropriate to the data in fig. 204 of Allen (1973), e.g. $\mu_1 = 1200$, $\mu_2 = 500$, $\sigma_1^2 = 160000$, $\sigma_2^2 = 22500$, is positively skewed. Using these values for mean and variance, 100 simulations of 60 ratios (close to the number of years in Allen's (1973) study) gave a mean skewness of 2.02, and all skewness values were positive. Allen's (1973) results should not, therefore, be considered informative about the shape of recruitment distributions.

Garrod (1983) fitted the Shepherd recruitment model individually to data for 11 fish stocks and estimated the standard deviations of the observations from the fitted curve. Because the number of observations was insufficient to describe the characteristics of the variation of recruitment of the individual stocks, he developed a method for combining the data. The deviation of each data point relative to the standard deviation of the appropriate stock was calculated and then aggregated over stocks to get a combined frequency distribution of relative deviations. From this analysis, he concluded that year class strength was lognormally distributed about the expected value. Because relative deviations are aggregated from a number of

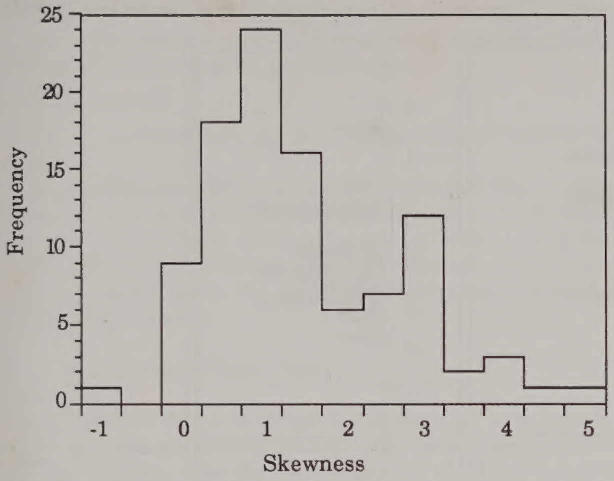


FIG. 1. Frequency distribution of skewness values for 100 North Atlantic fish stocks reported in Myers et al. (1990).

stocks in order to draw a conclusion about lognormality, this study must be treated with some caution.

Hennemuth et al. (1980) examined the null hypothesis that the recruitment data for each of 20 fish stocks represent a sample of yearly data drawn from a normally distributed population. The null hypothesis was rejected ($\alpha = 0.05$) for 12 cases (60%). Once the data were log-transformed, the null hypothesis could only be rejected for one stock at the same significance level. Adopting a similar approach, Myers et al. (1990) assembled recruitment data for 100 North Atlantic fish stocks and estimated the Pearson correlation coefficients between the normal and sample quantiles for the logged recruitment values. They found that the majority of the data sets were not significantly different from a normal distribution at $\alpha = 0.05$. In a reanalysis of the same data using the Shapiro and Wilk (1965) test for normality and setting H_0: recruitment values are a random sample from a normal distribution, it was found that the H_0 could be rejected in 68 cases at $\alpha = 0.05$. Setting H_0: log-transformed recruitment values are drawn randomly from a normal distribution, the H_0 could be rejected in 19 cases at $\alpha = 0.05$.

Although the results of Hennemuth et al. (1980), Myers et al. (1990), and the analysis carried out above suggest that lognormality occurs, estimates of recruitment reflect the natural variability of year class strength, with estimation error superimposed. If this error is multiplicative, for example of the form

(2) $\hat{R} = R\,e^{Z}$

where Z is normally distributed with mean zero and variance σ_2^2, then estimated recruitment will be lognormally distributed for R constant. This form of error is frequently assumed in trawl survey estimates (e.g. Peterman and Bradford 1987). Further, estimates of numbers at age from virtual population analysis may also be positively skewed and have greater density in the tail regions (larger value of kurtosis) than would be expected if the estimates were samples from a normal distribution, as a result of error in inputs (e.g. Sampson 1987). To illustrate the problem of error, the skewness of the recruitment distributions for the 100 North Atlantic stocks are compared with time-series of normally distributed recruitment values estimated with error.

The frequency distribution of skewness estimates obtained by analysing the data in Myers et al. (1990) for North Atlantic stocks is shown in Fig. 1. As indicated above, the majority of the stocks have recruitment distributions which are positively skewed. A second set of 100 recruitment time-series of equivalent lengths to those for North Atlantic fish stocks was generated from (2) with R drawn randomly from $N[1, 0.09]$ (a normal distribution with $\mu = 1$ and $\sigma^2 = 0.09$) and Z drawn randomly from $N[0, 0.09]$. While arbitrary, these distributions represent plausible recruitment variability and estimation error. The frequency distribution of skewness values for the generated series is shown in Fig. 2. It is apparent that most of the observed skewness values could be accounted for by estimation error if the above assumptions hold. Because of the potential effect of estimation error, empirical analyses cannot be interpreted as unequivocal evidence that the underlying distribution of recruitment is generally lognormal.

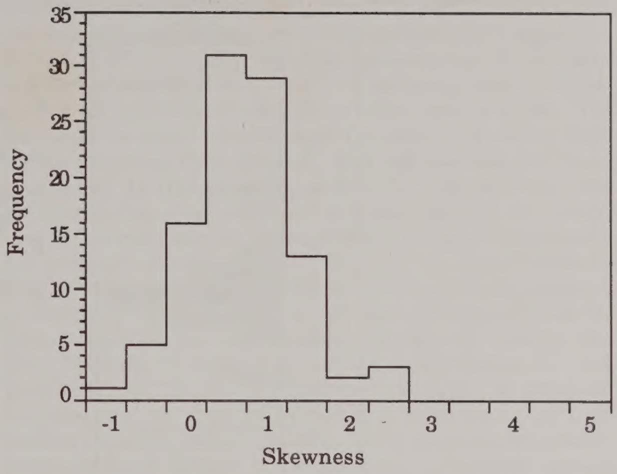


FIG. 2. Frequency distribution of skewness for 100 simulated series of equivalent lengths as those for the North Atlantic, generated from normally distributed recruitment estimated with lognormally distributed multiplicative error (see text for details).

The skewness of recruitment distributions by species for several stocks of Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), Atlantic herring (*Clupea harengus*), redfish (*Sebastes* spp.), American plaice (*Hippoglossoides platessoides*), and pollock (*Pollachius virens*) calculated from data in Myers et al. (1990) is plotted in Fig. 3. In the case of one haddock stock, data are included separately for two time segments. Data for two redfish stocks with skewness values less than 1 are considered suspect (R. Myers, Department of Fisheries and Oceans, St. John's, Nfld., pers. comm.). Cod, haddock, and herring time-series are of comparable lengths. Comparison is therefore valid and suggests that cod recruitment distributions may generally be less skewed than those of haddock and herring. The redfish data include stocks with high values of skewness despite generally short time-series whereas values for pollock and plaice are lower.

Theoretical Arguments for Lognormality

The Ricker recruitment model (Ricker 1954) has frequently been used to describe the relationship between spawners and subsequent recruits (e.g. Walters and Hilborn 1976):

(3) $R_t = S_{t-1} \exp(\alpha - \beta S_{t-1} + \upsilon_t)$

where R_t = recruits at end of generation t, S_{t-1} = spawners at the start of generation $t - 1$, α = a stock production parameter, β = equilibrium stock parameter (equilibrium stock in the

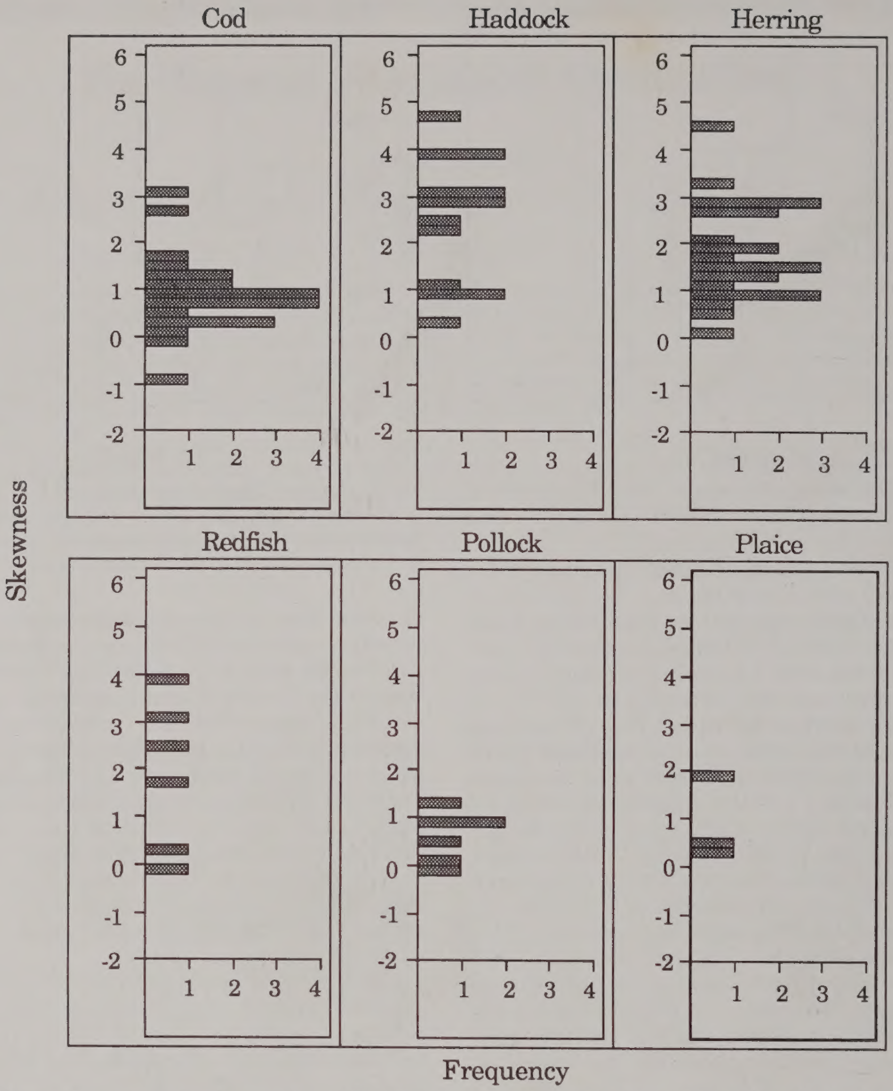


FIG. 3. Skewness values for recruitment distributions of North Atlantic fish stocks calculated from data in Myers et al. (1990).

absence of fishing is equal to α/β), and υ_t = random error, assumed to be normally and independently distributed with mean 0 and variance σ^2.

Because the error term υ_t in (3) is assumed to be normally distributed, recruitment is lognormally distributed. This model is mathematically convenient because it allows α and β to be estimated by least squares regression of $\ln(R_t/S_{t-1})$ against S_{t-1}, with normally distributed residual error. A theoretical argument for lognormality can be developed (Walters and Hilborn 1976; Peterman 1978) by noting that exp (υ_t) may be viewed as a random survival factor resulting from J independent, multiplicative survival rates s_j applied in succession to an initial population E of eggs, so that

$$(4) \quad R = E\, s_1 \cdot s_2 \cdot s_3 \cdot \ldots \cdot s_J.$$

Taking logarithms, one obtains

$$(5) \quad \ln R = \ln E + \ln s_1 + \ln s_2 + \ln s_3 + \ldots + \ln s_J.$$

If $s_1 \ldots s_J$ are independent random variates, then, as a consequence of the Central Limit Theorem, ln R will be approximately normally distributed, and therefore, R will be approximately lognormally distributed.

It must be noted that the proposition that lognormality is a consequence of (4) is only true if $s_1 \ldots s_J$ are independent, identically distributed random variates, J is large, and E is constant, conditions which are frequently overlooked and unlikely to occur. Further, instead of considering recruitment in terms of a series of survival rates applied to the set of all eggs, it may be informative to consider each egg or groups of closely associated eggs as having an independent probability of giving rise to a recruit or surviving one of several stages between spawning and recruitment (see Rothschild 1986). It will be shown below that the outcome taking this approach can be quite different from that obtained using (4).

Let p be the probability of surviving to recruitment and q the probability of not surviving to recruitment, so that

$$(6) \quad p + q = 1.$$

Each group of closely associated eggs can be thought of as an independent trial at successful recruitment, so that the mean number of recruits can be approximated by

$$(7)\quad R = p\,n\,E$$

where E = number of eggs in the group and n = number of groups.

Repeated independent trials are called Bernoulli trials if there are only two possible outcomes for each trial, success or failure, and their probabilities remain the same throughout the trials. The probability of obtaining exactly k successes from n Bernoulli trials is given by the probability density function for the binomial distribution:

$$(8)\quad \Pr\{k \text{ successes from } n \text{ trials}\} = \frac{n!}{k!(n-k)!}\,p^k q^{n-k}.$$

The properties of the binomial distribution are well known. Variance increases with increasing n and p, while skewness and kurtosis decrease. Further, with respect to (7), for a constant total number of eggs, variance increases with group size E at any value of p.

Typically, a spawner population consists of millions of females, each with annual fecundities in the hundreds of thousands, and only a small fraction of the eggs produced need to survive to ensure replacement. Consequently, although n will be large, p will be small. Note that the skewness and kurtosis values for $n = 100$ and $p = 0.01$ are the same as for $n = 1000$ and $p = 0.001$, so that examples below, which, for convenience, use n smaller than the annual fecundity of a spawner population, remain illustrative.

Now consider two cases: (i) a population produces 1×10^9 eggs, each with an independent probability $p = 1 \times 10^{-7}$ of surviving to recruitment (i.e. $n = 1 \times 10^9$, $E = 1$), and (ii) a population produces 4×10^7 groups of 25 eggs (i.e. $n = 4 \times 10^7$, $E = 25$), and each group of eggs has an independent probability $p = 1 \times 10^{-7}$ of surviving to recruitment, so that all individuals in the group either survive or die. The outcomes for these two cases were simulated by choosing 5000 values randomly from binomial distributions with the above parameters. In both cases the mean is 100, but in case (i) the distribution has small variance, skewness, and kurtosis values, and the normal quantile plot is close to a diagonal straight line for a normal distribution with the same mean and variance (Fig. 4) whereas in case (ii) the distribution has larger variance, is positively skewed, has a larger value of kurtosis, and the normal quantile plot departs from the diagonal straight line (Fig. 5). Case (i) represents survival independence — each individual egg has an independent probability of surviving whereas case (ii) represents survival dependence — all individuals in a group either survive or die in a dependent manner. From the above results, it is evident that a positively skewed distribution can result from a lack of independence in the probability of survival among individuals in a brood.

In cases (i) and (ii), p is constant for each group of eggs, fulfilling the requirements of the binomial distribution. If the binomial constraint is relaxed, alternative realisations can be explored. First consider a population which produces n groups of eggs, each of which experiences a random probability p_i of surviving to recruitment, where p_i is the product of J independent random probabilities p_j representing the interaction of environmental determinants of survival. The probability of the ith group of eggs surviving to recruitment is now given by

$$(9)\quad p_i = \prod_{j=1}^{j} p_{ij}.$$

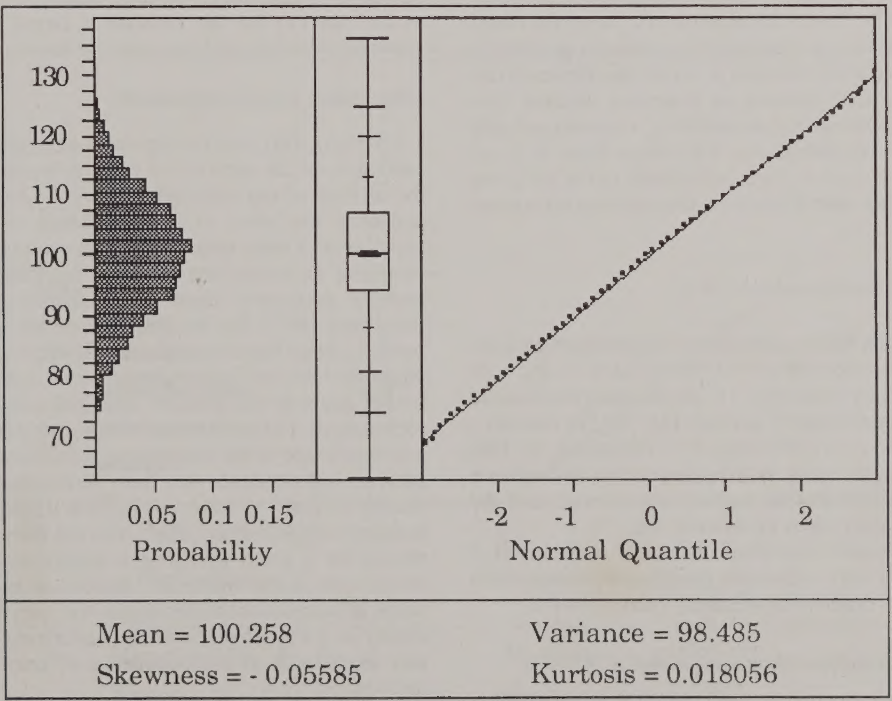


FIG. 4. Probability distribution, box plot, and normal quantile plot for values of recruitment from a population which produces 1×10^9 eggs, where each egg has an independent probability $p = 1 \times 10^{-7}$ of surviving (see text, case (i)). Note that the same vertical scale (recruitment) applied to all three plots. For comparison, the normal quantile plot shows the line for a normal distribution with the same mean and variance.

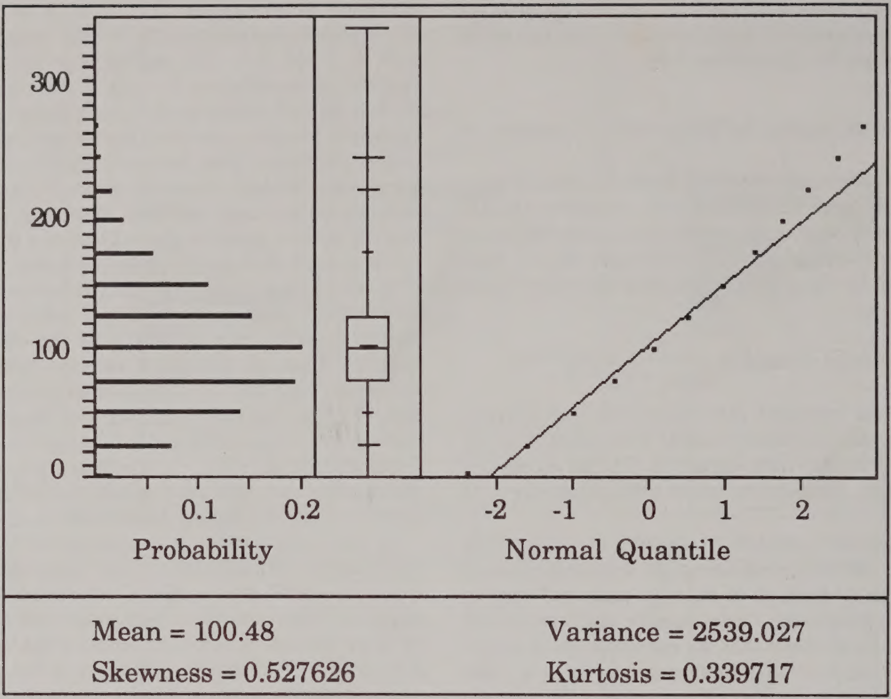


FIG. 5. Probability distribution, box plot, and normal quantile plot for values of recruitment from a population which produces 4×10^7 groups of 25 eggs, where each group has an independent probability $p = 1 \times 10^{-7}$ of surviving (see text, case (ii)).

Values for p_i can be simulated from the product of J probabilities drawn randomly from a distribution of p_j's, or the distribution of p_i's could be approximated by a suitable probability density function. Note that because p_i varies, the binomial distribution cannot be used. Instead, to determine whether individuals in the ith group survive, a variable z_i is drawn randomly from $U[0, 1]$ (uniform distribution with lower limit = 0 and upper limit = 1). If $z_i \leq p_i$, then individuals in the ith group survive to recruitment, else they die, so that recruitment is given by

$$(10) \quad R = \sum_{i=1}^{n} E_i \text{ conditional on } z_i \leq p_i.$$

For case (iii) in which 5000 realisations of recruitment are simulated from (9) and (10) with $n = 1 \times 10^4$, $J = 5$, $E_i = 1$, and p_j drawn randomly from $U[0, 1]$, the resulting distribution of recruitment is approximately normal (Fig. 6). For case (iv), which is identical to case (iii) except $n = 100$ and $E_i = 100$, mean recruitment is the same as for case (iii) but the variance is much larger, the distribution is positively skewed, and the distribution has a larger value of kurtosis (Fig. 7).

Now consider a population which produced n groups of E eggs, each of which has to survive a gauntlet of J independent survival episodes in order to successfully recruit, so that

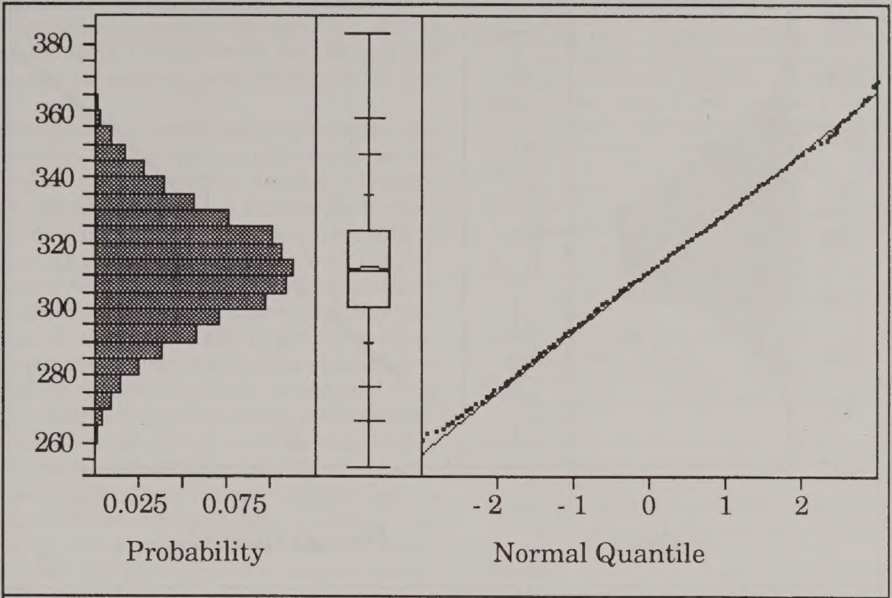
$$(11) \quad R = \sum_{i=1}^{n} E_i \text{ conditional on all } z_j\text{'s} \leq p_j\text{'s}.$$

For case (v) in which 5000 realisations of recruitment are simulated from (11) with $n = 10000$, $J = 10$, $E_i = 1$, p_j drawn randomly from $U[0.5, 1]$, and z_j drawn randomly from $U[0, 1]$, the resulting distribution of recruitment is again approximately normal (Fig. 8). In case (vi), which is identical to case (v) except $n = 100$ and $E_i = 100$, mean recruitment is the same as for case (v) but the variance is larger, the distribution is positively skewed, and the value for kurtosis is larger (Fig. 9).

Discussion and Conclusions

Cases (i), (iii), and (v) represent examples of survival independence — the survival of each offspring is independent of the survival of any other offspring in the brood. Survival independence can arise as a consequence of bet-hedging, the expression of traits selected through evolution in varying environments to reduce the variance in offspring, and thereby increase geometric mean fitness (Bulmer 1985; Seger and Brockman 1987). Bet-hedging fish, which spawn several independent, large batches of planktonic eggs (which disperse and therefore increase independence) over a prolonged spawning season and over an extensive spawning area, spread the risk of recruitment failure across many independent trials. The expected shape of the recruitment distribution for fish achieving survival independence has been demonstrated to be approximately normal. As most marine fish display a degree of bet-hedging in reproduction, there does not seem to be a compelling reason for a priori assuming a highly skewed distributional shape such as the lognormal. Instead, it is suggested that the shape of recruitment distributions will vary among stocks, primarily as a consequence of the underlying life history traits, and secondarily as a consequence of error in the estimation method.

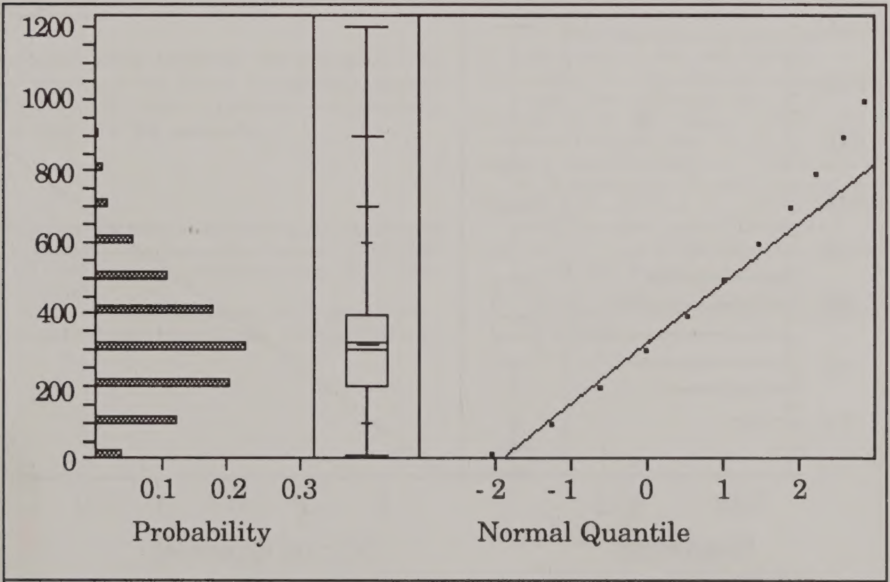
Estimates of the higher moments of a distribution may be associates with large uncertainty, so that comparisons should be made with care. Data in Fig. 3 are indicative of some species differences in skewness. A more detailed investigation which takes into account error in recruitment estimates and considers



Mean = 312.27 Variance = 309.506

Skewness = 0.01022 Kurtosis = 0.047171

FIG. 6. Probability distribution, box plot, and normal quantile plot for values of recruitment from a population which produces 1×10^4 eggs, where each egg has an independent probability p_i of surviving and p_i represents the interaction of several independent, random environmental determinants of survival (see text, case (iii)).



Mean = 318.78 Variance = 31551.62

Skewness = 0.615937 Kurtosis = 0.619425

FIG. 7. Probability distribution, box plot, and normal quantile plot for values of recruitment from a population which produces 100 groups of 100 eggs, where each group has an independent probability p_i of surviving and p_i represents the interaction of several independent, random environmental determinants of survival (see text, case (iv)).

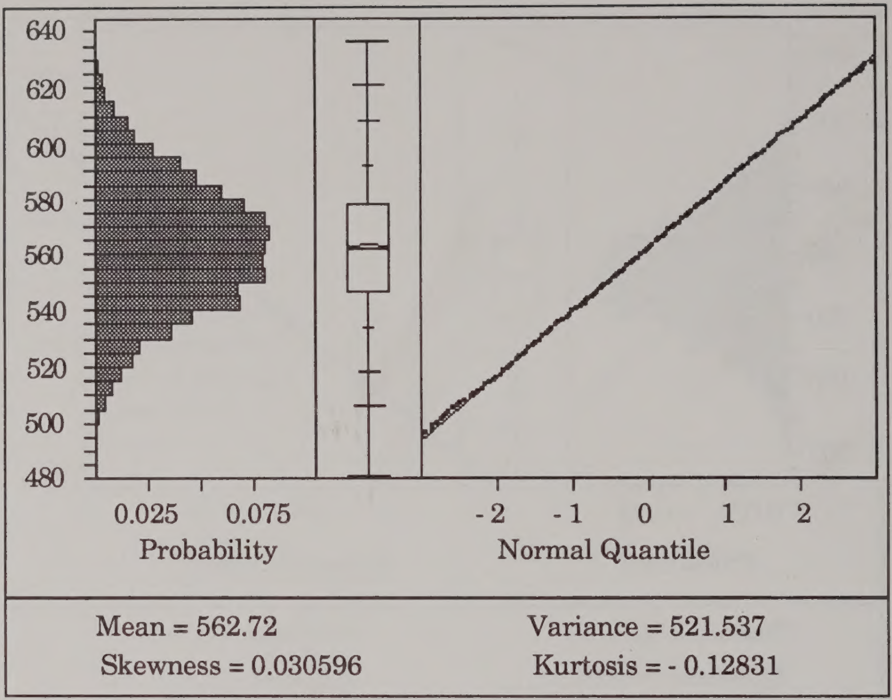


FIG. 8. Probability distribution, box plot, and normal quantile plot for values of recruitment from a population which produces 1 × 10^4 eggs, where each egg is subject to a gauntlet of independent random survival probabilities p_j (see text, case (v)).

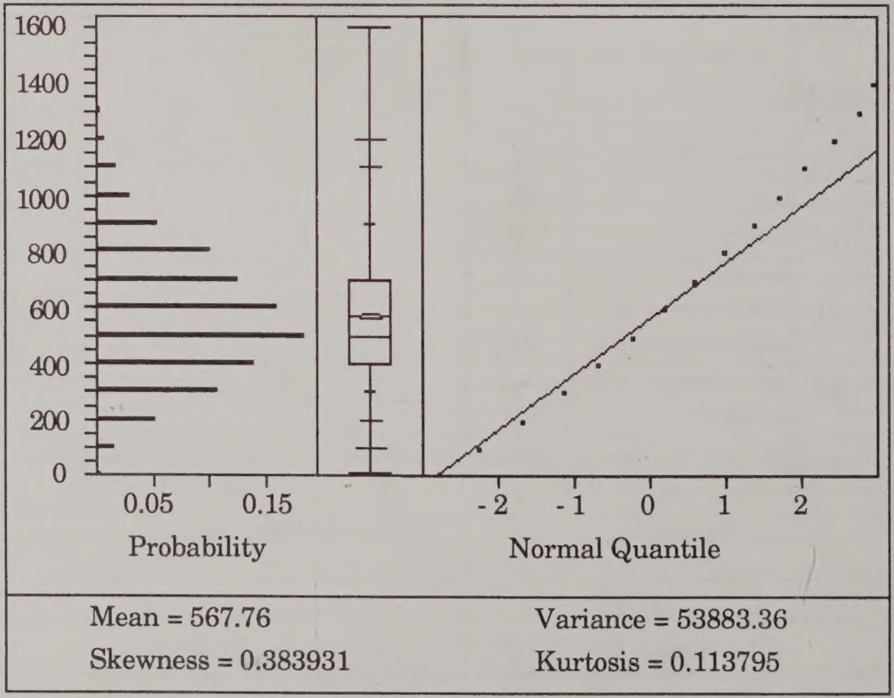


FIG. 9. Probability distribution, box plot, and normal quantile plot for values of recruitment from a population which produces 100 groups of 100 eggs, where each group is subject to a gauntlet of independent random survival probabilities p_j (see text, case (vi)).

differences, such as in fecundity and growth rates, among stocks within species groups would be required to more fully address the hypothesis that the shape of recruitment distributions will vary among stocks as a consequence of differences in life history traits.

Based on the theory presented here, a variety of distributional shapes can be expected to be descriptive of recruitment data for different stocks, and the most appropriate function to describe the shape should be selected on a stock by stock basis. Further, the shape of the distribution can be expected to change if there are changes in the expression of life history traits by the population (or if the error in the estimation method changes). Under heavy exploitation, there may be considerable reduction in population size, number of age classes, and geographical range, leading to less independence in survival among individuals in the brood and therefore to more positively skewed and more variable recruitment. Note that under the theory provided here, variance in recruitment is a function of survival independence rather than fecundity per se (cf. Fogarty et al. 1991). In moderately exploited populations, compensatory changes such as faster body growth rates and decreased age at maturity may counteract the loss of survival independence, thereby maintaining the symmetrical distribution and low variance associated with the recruitment of bet-hedging fish.

In conclusion, recruitment should be viewed as the outcome of a process in which individual reproductive products or groups of reproductive products have a probability of surviving. The shape of the resulting distribution of recruitment for a stock will depend on the amount of survival independence among individuals making up the year class. Thus, recruitment should be viewed as being closely linked to life history, and fisheries managers should be made aware of the possible effects of exploitation on the expression of life history traits.

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Size of the South American Sardine (*Sardinops sagax*) Population in the Northern Part of the Peru Upwelling Ecosystem after Collapse of Anchoveta (*Engraulis ringens*) Stocks

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Patterson, K. R., J. Zuzunaga, and G. Cárdenas. 1992. Size of the South American sardine (*Sardinops sagax*) population in the northern part of the Peru upwelling ecosystem after collapse of anchoveta (*Engraulis ringens*) stocks. Can. J. Fish. Aquat. Sci. 49: 1762–1769.

New estimates of sardine (*Sardinops sagax*) biomass suggest that a change in state of the Peruvian upwelling ecosystem, previously thought to be from one of anchoveta (*Engraulis ringens*) dominance to one of equal abundances of sardine and anchoveta, has been a clear switch in dominance from anchoveta to sardine. The sardine stock inhabits the northern part of the Peru upwelling system and extends its distribution into waters off Ecuador. Virtual population analyses show the population to have been considerably larger than was previously thought, with a biomass in 1982 estimated at between 15 and 18 million tonnes. The anchoveta collapse appears to have allowed a large bloom in sardine abundance. Sardine stock size has declined in recent years, concurrently with estimated mortalities in excess of $F_{0.1}$ and declining recruitment.

De nouvelles estimations de la biomasse du pilchard du Chili (*Sardinops sagax*) portent à croire qu'une modification de l'écosystème de remontées d'eau profonde du Pérou, soit que la dominance de l'anchois du Pérou (*Engraulis ringens*) aurait fait place à une abondance égale de pilchard et d'anchois, était réellement la dominance du pilchard sur l'anchois. Le stock de pilchard fréquente le secteur septentrional du système de remontées d'eau profonde du Pérou; son aire de répartition se prolonge dans les eaux hauturières de l'Équateur. Des analyses virtuelles de population révèlent que le pilchard était beaucoup plus abondant que l'on croyait; ainsi, sa biomasse en 1982 atteignait entre 15 et 18 millions de tonnes. L'effondrement du stock d'anchois semble avoir favorisé une poussée importante de l'abondance du pilchard. Au cours des dernières années, le stock de pilchard s'est appauvri en même temps que l'on notait un taux de mortalité estimatif supérieur à $F_{0,1}$ et une baisse du taux de recrutement.

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The fishery for Pacific sardine (*Sardinops sagax*) between 13 and 1°S on the eastern Pacific coast is of considerable economic importance. Catches from this stock averaged around 500 000 tonnes (t) in Ecuador and 2 million t in Peru over the period 1982–89. This importance is, however, a recent phenomenon, as prior to the collapse of the anchoveta (*Engraulis ringens*) fishery in 1972, catches were relatively low (Table 1).

Stocks of sardines, as of other clupeids, tend to have highly variable populations with more or less prolonged periods at a very high or a very low abundance (Csirke 1988) which have been called "régimes." Evidence of this phenomenon in *Engraulis*, *Sardina*, and *Sardinops* was reviewed by Lluch-Belda et al. (1989). For example palaeosediment analyses by De Vries and Pearcy (1982) have shown that sardine populations in the Peru upwelling area have undergone changes in abundance at around 100-yr intervals. In Japan and in the Benguela currents, it has been observed that where stocks of sardine and anchovy coexist, the stock of one species tends to increase dramatically on the collapse of the stock of the other (Lluch-Belda et al. 1989). These authors also presented evidence that climatic changes play a role in the balance between sardine and anchovy, as trends in solar radiation and sea and air temperatures are correlated with sardine catches in the various current systems.

There is evidence that the sardine stock in the Peru upwelling system boomed after the anchoveta stock collapsed in 1972. Before this collapse in 1972, the anchoveta stock dominated the ecosystem, and the abundance of sardine was low (Muck et al. 1987). Afterwards, a number of authors concluded that sardine stocks increased rapidly, and for some years anchoveta and sardine maintained similar stock sizes (Walsh 1981; Zuzunaga 1985; Villavicencio and Muck 1985; Pauly 1987; Jarre et al. 1989; Muck 1989).

New estimates of the sardine stock size in the 1980s are made here to supplement existing estimates from hydroacoustic surveys. To date, and in spite of the considerable commercial importance of the population of *S. sagax*, relatively little is known of its population biology in the Peru–Ecuador area, in contrast with well-documented population studies in other areas (MacCall 1979; Murphy 1966; Serre and Zuleta 1982; Salazar

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TABLE 1. Catches (thousands of tonnes) of *S. sagax* and other small pelagic fish in Ecuador and Peru. Sources: Peru, Instituto del Mar del Peru; Ecuador, Patterson et al. (1990). Dashes = no data available.

	Peru					
	S. sagax				Ecuador	
Year	3–13°S	S of 13°S	Total	Pelagics total	*S. sagax*	Pelagics total
1970	—	—	0.5	12357	—	—
1971	—	—	6	10381	—	—
1972	—	—	6	4548	—	—
1973	—	—	132	1801	—	—
1974	—	—	73	3870	—	—
1975	—	—	63	3213	—	—
1976	—	—	175	4143	—	—
1977	—	—	871	2228	—	—
1978	1174	84	1258	2949	—	—
1979	1525	202	1727	3400	—	—
1980	1038	458	1038	2398	—	—
1981	891	312	1203	2490	255	1073
1982	1391	120	1511	3318	314	1158
1983	865	182	1047	1417	104	547
1984	2560	287	2847	3107	649	1343
1985	2682	388	3070	3896	1216	1999
1986	1640	63	1703	5302	590	1300
1987	2230	92	2322	4325	210	754
1988	3400	66	3466	6352	382	950
1989	2530	46	2576	6488	261	691
1990	2369	34	2403	5535	18	168

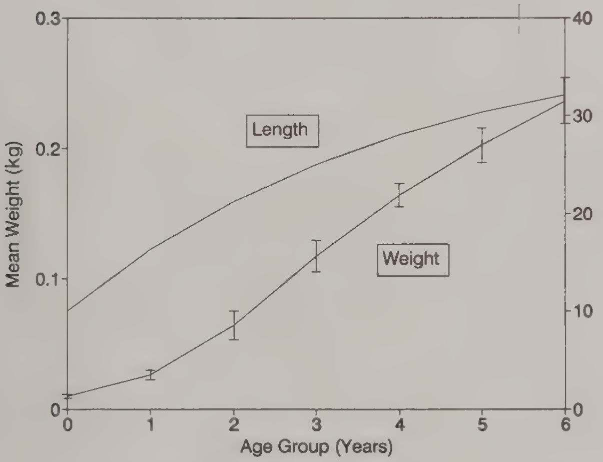

FIG. 1. Mean weight (± 1 SD) and length at age of *S. sagax* in landings in Peru over the period 1982–90.

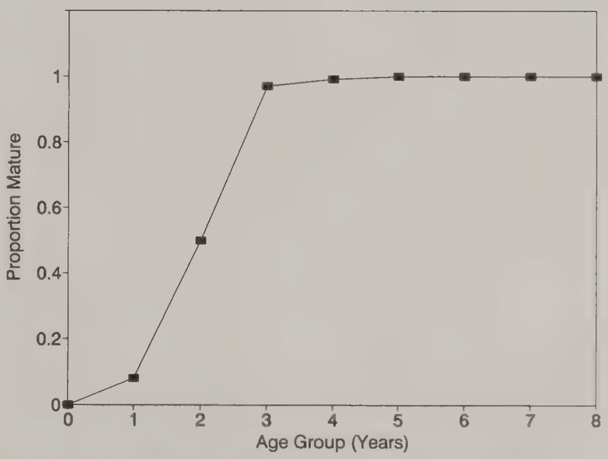

FIG. 2. Mean maturity ogive for *S. sagax* in Peruvian waters. Fish taken as "mature" are in maturity stages higher than III on the scale of Arriaga et al. (1983) i.e. gonads two thirds or more of body cavity volume).

1984; Serra 1986; Martínez et al. 1987; Crawford et al. 1983). Stock assessments have been made primarily by hydroacoustic surveys, which are principally designed to assess the anchoveta stock.

Methods

In order to verify assumptions about population changes in the ecosystem, age-based population analysis methods have been used to recalculate historical stock sizes of sardine. A sufficiently long time-series of catch at age data is now available from the fisheries of Ecuador and Peru to allow conventional methods to be used. These data have been generated by the routine landings sampling programmes of the Instituto Nacional de Pesca, Ecuador (Menz and French 1982), and the Instituto del Mar del Perú (Tables 2 and 3; Fig. 1 and 2). Otolith readings were made with reference to criteria established by Saldaña (1983), Dawson (1986), and Cárdenas (1987).

Sampling coverage has not been as good as one would expect in fisheries in more developed countries. Table 4 shows the history of sampling coverage in the Ecuadorean fishery, which was of the order of 0.3–0.7 samples per 1000 t landed. In Peru, landings are made in units normally under 200 t, and samples are taken from at least 5% of the landings by weight annually.

TABLE 2. Catches (millions at each age) of *S. sagax* in Ecuador, 1982–89. Source: Instituto Nacional de Pesca, Ecuador.

	Year								
Age	1982	1983	1984	1985	1986	1987	1988	1989	1990
0	0	0	0	0	0	0	0	0	0
1	28	0	89	2	78	1	17	82	4
2	277	25	245	194	321	17	17	455	75
3	380	216	816	2443	1231	413	369	76	7
4	526	278	1579	3878	1967	740	1273	466	25
5	302	123	828	1303	265	209	481	474	32
6	103	16	170	270	28	29	144	125	10
7	25	1	9	43	1	3	1	0	0
8+	0	0	0	0	0	0	0	0	0
Total	1641	659	3736	8133	3891	1412	2302	1678	153

TABLE 3. Catches (millions at each age) of *S. sagax* in Peru, north of 13°S. In this area, catches are assumed to consist overwhelmingly of northern stock *S. sagax*. Source: Instituto del Mar del Peru.

	Year								
Age	1982	1983	1984	1985	1986	1987	1988	1989	1990
0	11	9	575	23	0	86	150	9	2373
1	861	129	7088	1296	1	134	25218	1450	3676
2	337	84	4819	1944	7	130	6268	1018	763
3	568	273	3607	4693	744	595	1411	2308	807
4	1464	915	2754	5216	3983	4111	4654	4980	5197
5	3048	1886	3211	2983	2898	5333	5663	5066	4152
6	1149	1051	1538	1555	1297	2046	1389	1736	1947
7	493	290	273	330	229	455	160	101	141
8+	41	14	10	3	8	8	6	3	0
Total	7972	4651	23875	18043	9167	12898	44919	16671	19056

TABLE 4. Numbers of samples taken, fish measured, and otoliths read in Ecuador per 1000 t of catch.

Year	Samples	Fish measured	Otoliths read
1982	0.33	19.00	2.25
1983	0.52	54.47	9.23
1984	0.56	41.86	3.43
1985	0.31	29.96	1.62
1986	0.57	40.70	1.93
1987	0.68	56.25	2.95
1988	0.48	40.53	1.95
1989	0.22	27.52	2.00
1990	0.61	131.94	8.89

This implies a minimum sampling intensity of approximately 0.5 samples per 1000 t per year.

The lack of suitable data series for tuning, such as effort or reliable abundance estimates, causes some problems in the analysis, as conventional effort-tuned virtual population analysis (VPA) cannot be used. However, a VPA can be fitted to the data if certain constraining assumptions are made. Two possibilities are explored here: (1) to assume mean fishing mortalities, F, in the most recent year and (2) to force the best fit to a constant selection pattern. Both methods have their disadvantages. Assuming mean Fs leads to a loss of information about trends within the time period. However, the estimates of mean F and biomass within the period will tend to be robust to errors in the tuning procedure. The second option is to use a model in which a constant exploitation pattern is assumed and to tune the terminal F to obtain the best fit to the data. Results generated using this approach rely on the assumption that a constant selection pattern underlies the data. This may well not be the case in a pelagic fishery, as fish tend to migrate in size-dependent shoals, and the migrations, and thus the catchability of these size groups, depend on prevailing oceanographic conditions. Using this approach could well lead to misleading results if selection pattern does vary. In consequence, both tuning methods were used for comparative purposes.

The "mean conditions" fit was made by assuming constant effort in Peru and tuning by iteratively setting catchabilities in the last year to the geometric mean catchabilities over previous years. Terminal F in the last ages was set to 0.6 times the mean of F at the two previous ages, on account of a low selection on older ages estimated using separable virtual population analysis (Pope and Shepherd 1982).

The "constant selection" fit was made using a series of separable VPAs with a range of different starting values for terminal selection and for terminal F on the reference age group, F_{ref}. The final choice of F_{ref} and selection was made by seeking low and consistent residuals, a reasonably flat-topped exploitation pattern, and a consistent trend in fishing mortality.

Natural mortality, M, has been taken as 0.4, according to estimates by most other workers on this species (Murphy 1966; Serra and Zuleta 1982; Salazar 1984) and related species (Anonymous 1989).

As no independent data were available for tuning, the fitting procedures used here are rather arbitrary. It is appropriate to test the sensitivity of the analysis to decisions made about M, terminal F, and selection. In order to reduce the dimensionality of the problem, the effect of changing F in the last year was

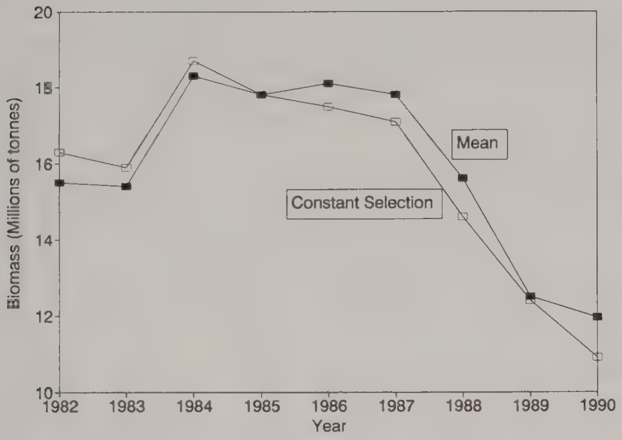

FIG. 3. Estimates of total stock biomass of *S. sagax* over the period 1982–90 using either mean log catchability in 1990 (solid squares) or assuming a constant selection pattern (open squares).

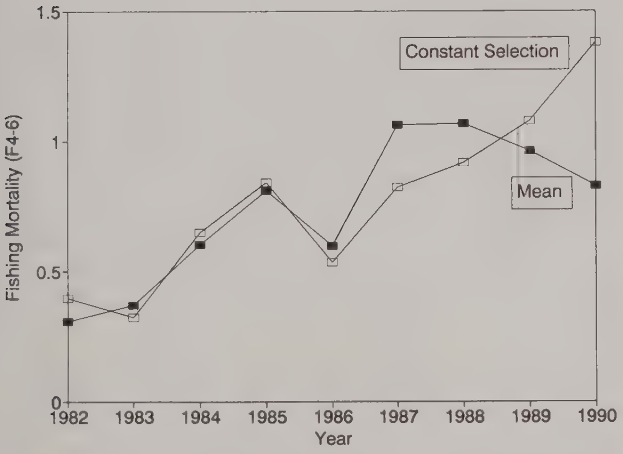

FIG. 4. Estimates of *F*. on *S. sagax* over the period 1982–90 using either assumed average values in 1990 (open squares) or assuming a constant selection pattern (solid squares).

removed by only considering values in the converged part of the analysis, i.e. before 1986. The analyses were repeated for a wide range of *M* and for different values of selection. This allows some estimation of the robustness of the conclusions drawn from the analysis wih respect to decisions made about the input parameters.

Results

The two methods yielded similar estimates of biomass and *F* (Fig. 3 and 4). They differ principally in that in the constant selection fit a steady increasing trend in *F* is derived, whilst in the mean conditions fit this trend is reversed as the terminal *F* is brought down again to mean levels (Table 4). The biomass estimates are very similar, both methods suggesting a decline since 1986, with the constant-selection model suggesting a slightly faster decline than the mean conditions fit. Detailed results of the mean conditions fit are given in Table 5, and a summary of the difference between the two fits is given in Table 6.

One may consider whether the higher *F* value in 1990 suggested by the constant selection model is likely to be a real phenomenon. The trend in *F* in the mean model is an overall gradual increase from 1982 to 1987 with a steady or declining trend from 1988 onwards. The latter decline is of course a consequence of the tuning method as the terminal *F* is brought down to the geometric mean of previous years. Since there were no reports of sudden cessation of fishing activity, one would instead expect a continuation of this trend up to the last years of the analysis. Consequently, the case of a regular trend in *F* (assumed in the constant selection model) is considered more likely than an unexplained decrease in *F* in the last years that is fitted by the mean conditions model. However, neither VPA adjustment contains information that allows terminal *F*s to be unambiguously determined.

Estimates of recruitment show a declining trend. Recruitment estimates are only reliable prior to 1985, as cumulative *F* on this cohort exceeded 1.3, indicating that the VPA is reasonably well converged on this group. These estimates suggest a declining trend in both stock and recruitment, whichever of the two model fits is used. The stock may presently be heading towards a recruitment collapse. Indices of relative recruitment (Lockwood 1987) were calculated for each year and from each analysis using estimated stock sizes at age 0 (Fig. 5). This index takes the value zero when recruitment balances deaths in the stock in any one year, is positive when recruitment exceeds deaths, and is negative when deaths exceed recruitment. The results from both analyses show a very similar tendency: recruitment shows a declining trend to 1985.

The analyses were repeated using different values of *M* and terminal selection. Closely similar results were obtained using either the constant selection or the mean conditions fit, and only results from the former are presented (Fig. 6). This shows that choices made about selection had a negligible effect on the biomass estimates for 1985. Choice of *M* was more important, yet even forcing obviously unreasonable natural mortality values of *M* (e.g. 0.05), the estimate of sardine biomass still remained substantially above the estimated anchoveta biomass. Over the likely reasonable range of *M* (0.3–0.5), sardine biomass greatly exceeds anchoveta biomass.

Discussion

The estimate of sardine biomass from the VPAs exceeds the estimates from hydroacoustic surveys over the typical range of ages encountered in the survey catch samples (Table 7). These show that the hydroacoustic survey estimates are considerably lower than either of the VPA estimates.

Sardines are likely underrepresented in the hydroacoustic surveys, as the surveys are designed principally to target anchoveta stocks. Juvenile fish may not be well represented in the surveys on account of having an inshore distribution. There is some anecdotal evidence to support this. However, independent estimates of stock abundance correspond well with the estimates made here. Carrasco et al. (1991) estimated the spawning stock biomass of sardine at 3.2 million t, a value close to the present estimates. It is not appropriate to discuss the methodology of the hydroacoustic surveys here, but a discussion of some sources of uncertainty in the present assessment follows.

Sampling intensity on this fishery has not reached levels normally considered desirable for age-structured assessments. A useful rule of thumb to assess adequacy of sampling is that a coverage in excess of one sample per quarter and per 1000 fish

TABLE 5. Summary of results using the mean conditions fit with effort assumed constant. Note that mean F does not equal 1990 F at age because the former is an arithmetic mean and the latter a geometric one.

Age	1982	1983	1984	1985	1986	1987	1988	1989	1990	Mean
	Fishing mortality (F) at age									
0	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.02	0.00
1	0.01	0.00	0.10	0.02	0.00	0.00	1.22	0.03	0.03	0.02
2	0.02	0.00	0.10	0.05	0.01	0.00	0.14	0.24	0.02	0.03
3	0.05	0.02	0.15	0.24	0.07	0.03	0.07	0.09	0.25	0.09
4	0.19	0.09	0.31	0.64	0.40	0.30	0.35	0.41	0.38	0.32
5	0.69	0.37	0.67	0.76	0.63	1.12	1.03	0.86	0.85	0.71
6	0.56	0.65	0.82	1.02	0.75	1.76	1.82	1.61	1.26	0.93
7	0.38	0.30	0.45	0.54	0.42	0.87	0.85	0.74	0.63	0.49
Mean	0.24	0.18	0.32	0.41	0.28	0.51	0.69	0.50	0.43	0.32
	Stock biomass at age and year (thousands of tonnes)									
0	1487	1411	836	923	1231	668	1203	1924	1268	1217
1	2844	2702	2772	2338	1681	2121	1003	1199	2293	2106
2	2620	3385	3583	3093	4791	3098	3165	638	2308	2965
3	3100	3136	4256	4533	4766	5110	3227	3645	560	3593
4	2342	2430	3335	3716	3253	3894	4213	3082	3224	3277
5	1650	1451	2254	2002	1511	1953	2212	2197	1692	1880
6	830	560	976	839	673	699	483	590	687	704
7	573	328	297	318	205	258	89	64	88	247
8+	55	19	13	3	9	5	4	2	0	12
Total	15502	15421	18322	17765	18120	17806	15598	13341	12119	16001
	Stock number at age and year (thousands of millions)									
0	148.7	138.3	126.6	103.7	125.6	62.4	97.0	194.4	126.8	124.8
1	88.9	99.7	92.7	84.4	69.5	84.2	41.8	63.8	130.3	83.9
2	44.7	58.9	66.7	56.3	55.5	46.5	56.3	8.3	41.5	48.3
3	25.1	29.5	39.4	40.6	36.0	36.9	31.1	32.7	4.4	30.6
4	14.0	16.0	19.4	22.8	21.5	22.5	23.9	19.4	20.0	19.9
5	7.9	7.8	9.8	9.5	8.1	9.6	11.2	11.3	8.6	9.3
6	3.5	2.7	3.6	3.3	3.0	2.9	2.1	2.7	3.2	3.0
7	2.0	1.3	0.9	1.1	0.8	0.9	0.3	0.2	0.4	0.9
8+	1.6	0.6	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.3
Total	335.0	354.3	359.1	321.7	319.9	266.1	263.7	332.7	335.1	321.0

TABLE 6. Summary of results from the two analyses. Mean of values over the period 1982–87.

Parameter	1[a]	2[b]
Spawning stock biomass (millions of tonnes)	5.2	5.4
Total biomass (millions of tonnes)	17.2	17.2
Recruitment (billions)	11.7	11.5
Mean F at age		
0	0.0013	0.0012
1	0.0223	0.0227
2	0.0289	0.0286
3	0.0921	0.0901
4	0.3231	0.3122
5	0.7086	0.6645
6	0.9272	0.8044
7	0.4907	0.4475

[a] VPA tuned using mean log catchability in the last year.
[b] VPA tuned to fit constant selection pattern.

is necessary in order to achieve a coefficient of variation in the catch at age data below 10% (Stevenson 1983). This is the minimum level of precision below which age-structured projection models cannot reliably be used to set total allowable catches to maintain target levels of fishing mortality and represents a sampling intensity over eightfold greater than that currently being achieved in Ecuador and Peru. The coefficient of variation in the catch at age data presented and used here is probably considerably more than 10%. Some evidence of this can be seen in Table 5, which shows large fluctuations in F between cohorts and ages. However, no attempt was made here to make age-structured catch predictions, merely to analyse historical trends in stock size. These should be highly robust to a somewhat elevated coefficient of variation in the catch at age matrix.

Bias could have been introduced by ageing errors, but this is unlikely. An independent analysis of growth in sardine using an analysis of modal progressions gave growth parameters close to those estimated from conventional ageing, strongly suggest-

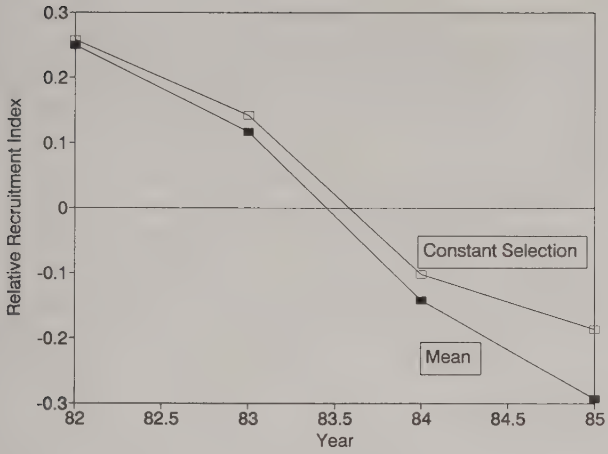

FIG. 5. Relative recruitment index, according to Lockwood (1987), for *S. sagax* in Ecuador and Peru using data from the mean log catchability adjustment (solid squares) and the constant selection model (open squares).

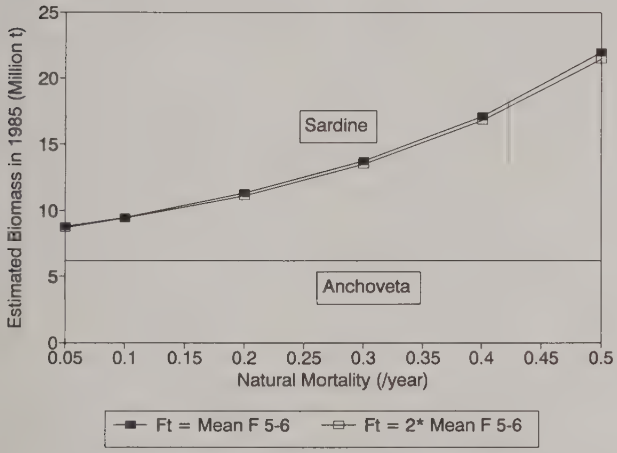

FIG. 6. Estimates of sardine stock biomass in 1985 from repeated VPAs using a range of values for *M* and selection on the oldest age.

TABLE 7. Comparison of VPA and survey biomass estimates (millions of tonnes) for northern stock *S. sagax* in Peruvian waters. Hydroacoustic data from Vilchez et al. (1988); egg production biomass estimate from Carrasco et al. (1991).

	Sardine							
		VPA estimates						
		Biomass ages 3+		Spawning biomass				
Year	Acoustic survey Peru	1[a]	2[b]	1[a]	2[b]	Egg survey estimate	Anchoveta, acoustic survey Peru
1981							3.00
1983	4.84	8.5	8.2	4.6	4.8		
1984	4.13	11.1	11.4	4.6	4.7		1.50
1985	2.10	11.4	11.7	4.5	4.7		6.21
1986	2.36	10.4	10.7	6.0	6.2		2.86
1987	3.80	11.9	11.9	6.9	7.0		1.05
1988		10.2	9.6	4.9	4.7		6.00
1989		9.6	7.7	4.1	3.3		3.06
1990	5.7	6.3	6.0	3.7	3.0	3.2	

[a] Results from VPA tuned using assumed constant effort in Peru and mean log catchability in both countries in 1990.
[b] Results from VPA tuned towards constant selection pattern.

TABLE 8. Mean total lengths of samples of sardine taken by midwater trawl at various depths. From Ñiquen (1986).

Depth band (m)	Mean length (cm)	Catch rate (t/h)
0–50	28.0	4.1
50–100	27.8	4.0
100–150	27.6	4.9
150–200	28.8	4.1
>200	28.8	1.2

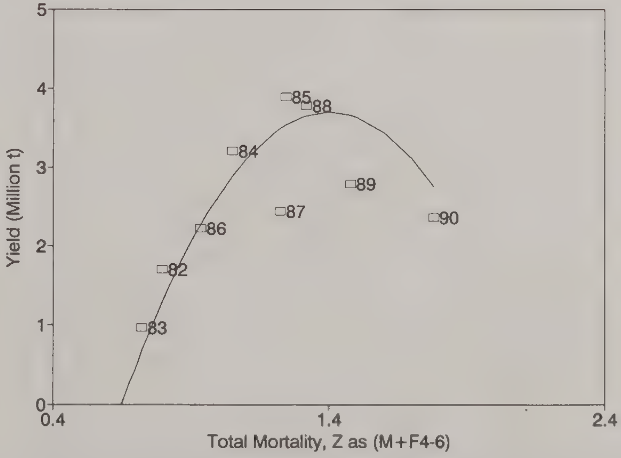

FIG. 7. Caddy and Csirke (1983) plot for the constant selection adjustment. Note that the descending limb of the parabola crosses the zero yield axis at *M*.

ing that the otolith reading method employed is correct (Cárdenas and Mendo 1985).

An additional problem is the lack of any suitable time-series of abundance or effort data that has made it impossible to make objective estimates of recent population sizes. The results of this analysis can only be considered entirely objective over the period 1982–85. However, the results of the sensitivity analysis show that the conclusion that sardine stock considerably exceeded the anchoveta stock in 1985 is quite robust to assumptions made in the analytic procedure. Additionally, some checks on the internal consistency of the model may be made. As the data on this stock are rather sparse and only a short time-series of data is available, such checks are desirable.

The separable VPA showed a low selection on older ages, and there is independent evidence that this is a real phenomenon. Ñiquen (1986) sampled from the midwater catches made by a Cuban stern trawler and found that fish in deeper water, which are inaccessible to the purse-seiners exploiting the stock, have mean total length 1 cm longer than fish in the surface waters (Table 8). Larger and older fish are therefore more likely to escape purse-seine fishing. However, the choice of terminal selection is not critical in the analysis.

A check of consistency of the estimate of *M* was made using a Caddy and Csirke (1983) plot. The descending limb of the eye-fitted paraobola is expected to cross the axis for zero catch at $M = 0.4$ (Fig. 7). Note, however, that in this plot the

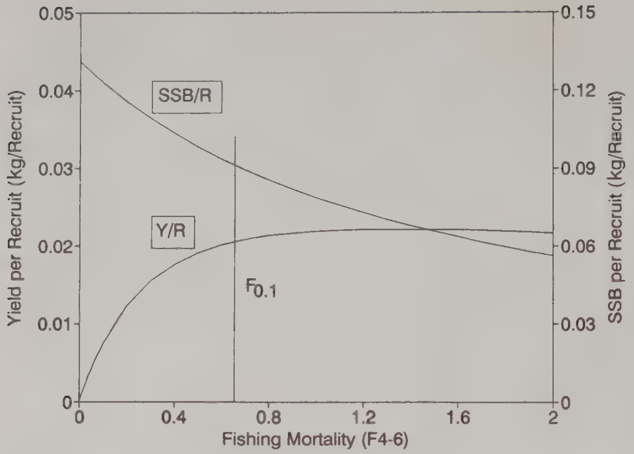

FIG. 8. Age-structured yield per recruit projection for the northern stock of *S. sagax* in the Peru upwelling system.

x-intercept is closer to 0.6. This discrepancy is unlikely to be due to an incorrect value of M used in the analysis, as choice of a higher M in a repeated analysis leads to an even higher Z-intercept, but rather suggests losses from the fishery due to emigration or escapement. This coincides with the observation of escapement of older fish noted above.

Results given here suggest that the northern stock of *S. sagax* in the Peru upwelling system has declined considerably in biomass over the period 1982–88. This decline appears to have been caused by a decrease in recruitment, which is very probably associated with a reduction in spawning stock biomass caused by fishing. From 1982 to 1985, recruitment was insufficient to replace losses through fishing and natural mortality. The decrease in biomass accompanied by a rapid increase in F is typical of a stock fished well in excess of sustainable levels.

Thompson and Bell (1934) age-structured yield per recruit projections were calculated for both the analyses. These failed to provide estimates of F_{max}, but $F_{0.1}$ was estimated at 0.62 and 0.64 from the constant selection and mean log catchability methods, respectively. The two curves were similar, and only that for the constant selection fit is given (Fig. 8). Estimates of $F_{0.1}$ at approximately 0.6 are consistent with the observation that both the Californian and Chilean sardine stock biomasses declined rapidly after F exceeded 0.5 (MacCall 1979; Martínez et al. 1989) and with the estimation of $F_{med} = 0.5$ in *Sardina pilchardus* in Europe (Anonymous 1989). According to either of the analyzes presented here, $F_{0.1}$ has been exceeded in each year since 1984, with the exception of 1986. The sardine stock off Peru and Ecuador is therefore assessed as being overexploited.

The present results show that sardine stock sizes have considerably exceeded previous estimates of anchoveta biomass (Table 7) in the period, so in fact a switch in dominance appears to have taken place. The present results show a decline in recruitment in sardine and suggest that the ecosystem may be reverting to its pre-1972 state. Muck et al. (1987) have shown that sardine predation on anchoveta eggs is likely to be an important factor regulating the population of the latter (although Smith et al. (1989) have reported contradictory results) so that the present reduced abundance of sardine may allow an increase in anchoveta biomass. A resurgence in the anchoveta stocks does seem to be underway (IMARPE, internal reports), although intensive fishing activities are likely to slow this recovery. The present decline of the sardine stock in the area may signal a return to pre-1972 conditions in the Peru upwelling ecosystem, with high anchovy biomass and low sardine stocks. The relatively high abundance of sardines over the period 1972–85 may have been a temporary interruption of a longer term régime.

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Allozyme Variation of Great Lakes Alewife, *Alosa pseudoharengus*: Genetic Differentiation and Affinities of a Recent Invader[1]

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Allelic frequencies for six polymorphic allozyme loci (27 loci examined), as determined by starch-gel electrophoresis, were not significantly different for alewife, *Alosa pseudoharengus*, among the Great Lakes (Ontario, Erie, Huron, and Michigan). Alewife from one of the Finger Lakes (Cayuga Lake) had allelic frequencies similar to Great Lakes alewife, and the alleles of Great Lakes alewife form a subset of those found in Cayuga Lake. In contrast, Maritime Canada populations (Gaspereau, Miramichi, and St. John rivers) were distinct from each other and from the Great Lakes and Cayuga Lake populations. Highly significant allelic frequency differences were observed among the Maritime populations and between the Maritime populations and the Great Lakes or Cayuga Lake populations. Fifteen alleles were not shared between the Great Lakes and the Maritime populations. We concluded from these results that Great Lakes alewife and Cayuga Lake alewife form a contiguous stock and that Great Lakes alewife are a recent invader that probably originated from the Hudson–Mohawk rivers via the Erie Canal and the New York Finger Lakes rather than the previously suggested alternative origin from the Canadian Maritimes via the St. Lawrence River.

Nous avons examiné chez le gaspareau, *Alosa pseudoharengus*, les fréquences alléliques pour six loci d'allozymes polymorphes (27 loci examinés) par électrophorèse sur gel d'amidon, et nous avons observé des différences non significatives parmi les populations des Grands Lacs (Ontario, Érié, Huron et Michigan). Les gaspareaux provenant de l'un des lacs Finger (lac Cayuga) présentaient des fréquences alléliques similaires à celles des gaspareaux des Grands Lacs, et les allèles des gaspareaux des Grands Lacs forment un sous-ensemble de ceux des poissons observés dans le lac Cayuga. Par contre, les populations des provinces Maritimes du Canada (rivières Gaspereau, Miramichi et Saint-Jean) étaient distinctes les unes des autres et de celles des Grands Lacs et du lac Cayuga. Nous avons observé des différences hautement significatives dans les fréquences alléliques parmi les populations des Maritimes et entre ces populations et celles des Grands Lacs ou du Lac Cayuga. On a compté quinze allèles qui n'étaient pas communs aux populations des Grands lacs et des Maritimes.. Nous concluons à partir de ces résultats que les gaspareaux des Grands Lacs et du lac Cayuga forment un stock continu, et que les gaspareaux des Grands Lacs n'ont envahi cette masse d'eau que récemment; ils provenaient probablement des rivières Hudson–Mohawk, via le canal Érié et les lacs Finger de l'État de New York, et non pas, comme on le proposait précédemment, de la région canadienne des Maritimes via le Saint-Laurent.

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The alewife, *Alosa pseudoharengus*, is generally considered to be a recent invader of the Great Lakes, although it has also been suggested that it is a relic of the colonization after the last glaciation (Radforth 1944). Two main routes have been suggested for the recent invasion hypothesis. One is via the Hudson–Mohawk rivers, Erie Canal, and New York Finger Lakes system, which is connected to Lake Ontario by several water routes such as the Oneida River. This theory is supported by the observation that alewife were present in two Finger Lakes, Cayuga and Seneca, before they were observed in Lake Ontario (Smith 1892). Bean (1884) first reported the presence of the alewife in Lake Ontario in 1873, and subsequently, large numbers were observed (Wright 1892). The second route of invasion suggested is from Maritime Canada via the St. Lawrence River. Alewife are presumed to have invaded the upper Great Lakes via the Welland Canal or the Lake Erie Barge Canal from Lake Ontario to Lake Erie, where they were first reported in the early 1930's. Subsequently, alewife have been observed in Lakes Huron, Michigan, and Superior in 1933, 1949, and 1954, respectively (Scott and Crossman 1973).

Within lakes Ontario and Michigan, alewife have proliferated and now comprise the principal prey of many salmonine species which support a significant sport fishery (Brandt 1986; Jude et al. 1987). Because of their abundance and behavior, alewife are susceptible to capture or impingement in the large volumes of cooling water drawn from the Great Lakes by industries such as thermal electric generating stations (Kelso and Milburn 1979). The concern has been raised that impingement and especially exploitation by salmonines may have negative

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local impacts on alewife stocks (Stewart and Ibarra 1991). The genetic structure of Great Lakes alewife populations could appreciably modify such impacts, i.e. selective mortality of fish in a population subdivided into discrete subunits would probably have a much greater effect on the population's genetic variability than would nonselective mortality of an equivalent number of fish from a large homogeneous population. Events that are associated with colonization such as founder effects (i.e. the colonizers are not genetically representative of the donor population) and population bottlenecks (i.e. the colonizers are temporarily reduced to very low numbers of breeders) can substantially reduce genetic variability and alter the genetic character of local stocks which would be reflected in allozyme variation.

In this study, we compared allozyme variation of alewife from four of the Great Lakes (Ontario, Erie, Huron, and Michigan), one of the Finger Lakes (Cayuga Lake), and three Maritime Canada populations (St. John, Miramichi and Gaspereau rivers). The results are discussed with respect to the origin and genetic structure of Great Lakes alewife and the potential impact of impingement and predation by salmonines on alewife populations.

Materials and Methods

Alewife were collected from Lake Ontario in 1986 at Cobourg, Pickering Nuclear Generating Station (NGS), and Grimsby; the Niagara River at Queenston in 1986; Lake Erie at Long Point Bay in 1991; Lake Huron at the Bruce NGS at Iverhuron in 1986 and South Baymouth in 1987; Lake Michigan at Saugatuck Bay in 1990; Cayuga Lake, New York State, in 1991; the Gaspereau River, Nova Scotia; the St. John River, New Brunswick, at the Mactaquac Dam; and from the Miramichi River at Millbank, New Brunswick, in 1987 (Fig. 1). At the Maritime locations, adult alewife were captured in trap nets during their spawning run (late May – early June). In Lake Ontario, the Niagara River, and Cayuga Lake, seine or dip nets were employed to collect yearling and older alewife during their spawning period (late May – early June). At Pickering, "non-impinged" alewife were collected from the nearshore waters of Lake Ontario at a beach approximately 1 km west of Pickering NGS "A" in June, while "impinged" alewife were collected within the cooling water intake at Pickering NGS "A" also in June. From Lake Erie, adults and juveniles were collected in late January. At South Baymouth, postspawning alewife were collected in July using gill nets. Alewife were collected at Bruce NGS "A" as young-of-the-year fish by seine in September. In Saugatuck Bay, Lake Michigan, adults were collected in late April.

Alewife from Lake Ontario, Queenston, and Bruce NGS "A" were transported live to our laboratory and then sampled for electrophoretic analysis. Fish from most other locations were frozen on dry ice following capture and then transported to our laboratory for sampling and analysis. The samples from Cayuga Lake, Lake Erie, and Lake Michigan were transported fresh on ice and processed immediately. Horizontal starch-gel electrophoresis was used to assay enzyme expression in liver and epaxial muscle tissue according to the procedure outlined by May et al. (1979). Buffer systems used followed Casselman et al. (1981). Enzyme names and locus abbreviations follow Shaklee et al. (1990). The enzymes, loci, and their international standard numbers assayed in this study were aspartate aminotransferase (_sAAT*_; 2.6.1.1), adenylate kinase (_AK*_; 2.7.4.3), creatine kinase (_CK-2*_; 2.7.3.2), cathodal muscle protein (_CMP*_), fructose-biphosphate aldolase (_FBALD*_; 4.1.2.13), glycerol-3-phosphate dehydrogenase (_G3PDH-1*_; 1.1.1.8), β-galactose aminidase (_GAM*_; 3.2.1.53), glyceraldehyde-3-phosphate dehydrogenase (_GAPDH-1*_, _GAPDH-2*_; 1.2.1.12), general muscle protein (_PRO-3*_), glucose-6-phosphate isomerase (_GPI*_; 5.3.1.9), 1-iditol dehydrogenase (_IDDH*_; 1.1.1.14), isocitrate dehydrogenase (_mIDHP*_, _sIDHP*_; 1.1.1.42), L-lactate dehydrogenase (_LDH-1*_, _LDH-2*_; 1.1.1.27), malate dehydrogenase (_mMDH*_, _sMDH-1*_, _sMDH-2*_; 1.1.1.37), malic enzyme (_mMEP-*_, _sMEP*_; 1.1.1.40), mannose-6-phosphate isomerase (_MPI*_; 5.3.1.8), 4-methylumbelliferyl phosphatase (_MUP*_; 3.1.3.2), phosphogluconate dehydrogenase (_PGDH*_; 1.1.1.44), phosphoglucomutase (_PGM-1*_, _PGM-3*_; 5.4.2.2), superoxide dismutase (_SOD*_; 1.15.1.1), and xanthine dehydrogenase (_XDH*_; 1.2.1.37). For the enzymes or proteins *CK*, *G3PDH*, *PRO*, and *PGM* for which more than one locus was detected, a numbering system analogous to that proposed for salmonids was used (Shaklee et al. 1990). _CK-1*_, expressed best in stomach muscle tissue, was not scored. _G3PDH-2*_ in liver, _PRO-1*_ and _PRO-2*_ in muscle, and _PGM-2*_ in liver were not scored because of low activity. For the frozen Maritime samples, St. John and Gaspereau rivers, we were unable to obtain consistent results for *IDDH* and, hence, for these two populations this system was not scored.

Genetic homogeneity within samples was assessed using estimates of deviation from Hardy–Weinberg proportions with chi-square goodness-of-fit tests. Differences in allelic frequencies between populations were tested with the likelihood ratio chi-square (Sokal and Rohlf 1981) or Fisher's exact test in cases where expected frequencies of some phenotypic classes were low. Clustering among populations was visualized using the unweighted pair-group method with arithmetic averaging (UPGMA) based on Nei's standard genetic distance (Nei 1972). Several other measures of genetic distance were also explored (arc and chord distance, Cavalli-Sforza and Edwards 1967; Rogers distance, Rogers 1972; Prevosti distance, Wright 1978). Total genetic variation among individual alewife was subdivided into regional contributions (Maritime versus Great Lakes and Cayuga Lake), within regions (i.e. among Great Lakes and Maritime populations), and within populations (i.e. among sampling sites within lakes) using Wright's fixation index, F_{ST} (Wright 1978). Most computations for the analyses of allelic variation were performed using the Biosys© computer program (Swofford and Selander 1989).

Results

Alewife were polymorphic at 13 of the 27 loci examined (Table 1). Four of these polymorphisms were for very rare alleles (_AK*53_, _mIDHP*150_, _mMEP*97_, and _LDH-1*-468_) with frequencies less than or equal to 0.01 in all populations sampled. Three deviations from Hardy–Weinberg proportions were observed: _IDDH*_ for the Grimsby, Lake Ontario, and Lake Erie samples with $P = 0.019$ and 0.012, respectively, and _PGDH*_ for the Miramichi sample with $P = 0.030$. The deviation for the Miramichi sample was due to a single unexpected rare homozygote in our sample of 100 fish. The _IDDH*_ deviations are about what is expected by chance at the 5% level of significance for the 53 independent samples tested. However, for 29 of these samples, the power of the test is very low because there were less than four observed heterozygotes or rare homozygotes per sample. The Maritime samples tended to have somewhat larger genetic diversity, defined as the proportion of polymorphic loci, the mean number of alleles per locus, and

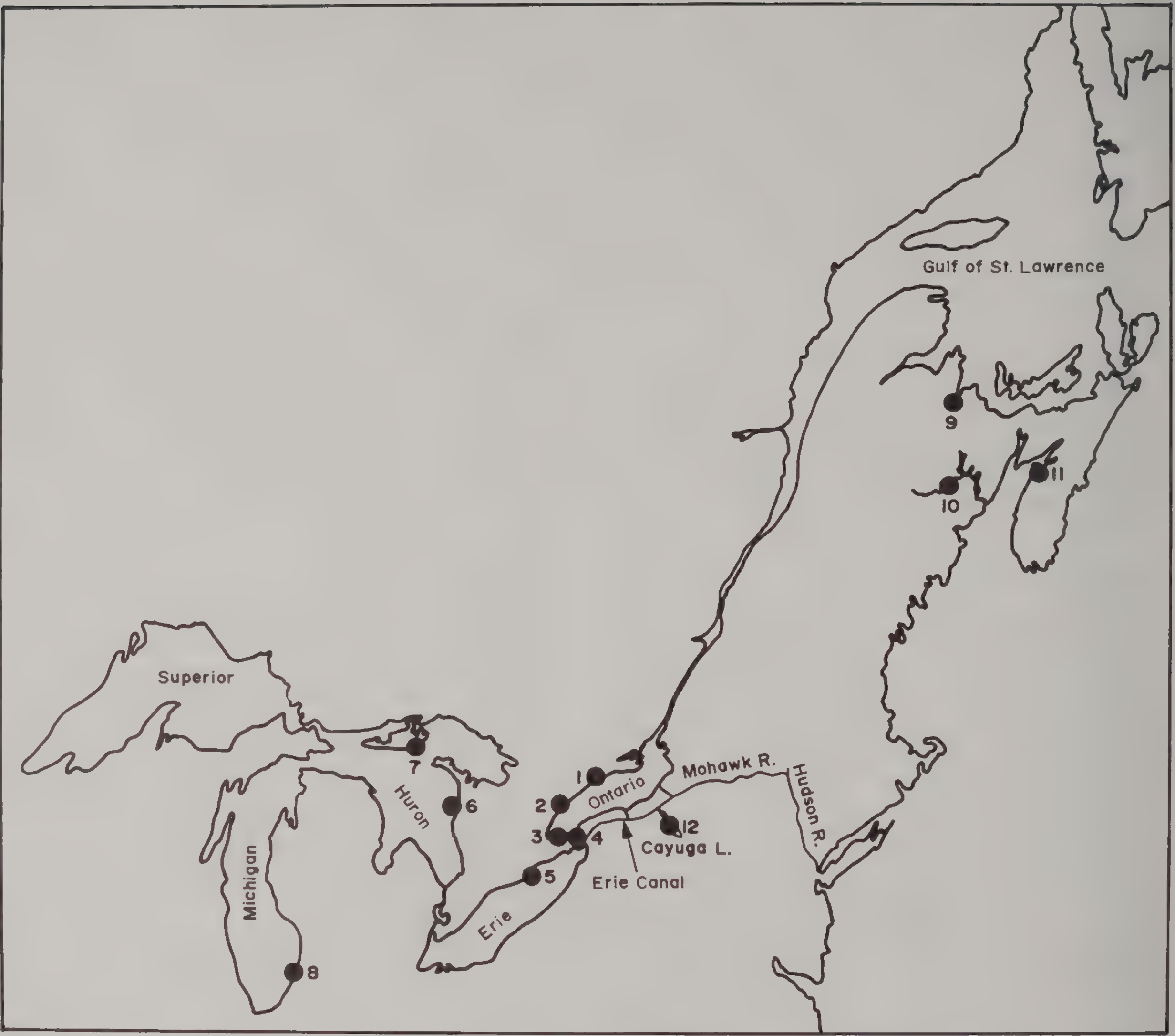

FIG. 1. Alewife collection sites: 1, Cobourg; 2, Pickering; 3, Grimsby; 4, Queenston; 5, Lake Erie; 6, Bruce; 7, South Bay; 8, Lake Michigan; 9, Miramichi River; 10, St. John River; 11, Gaspereau River; 12, Cayuga Lake.

the average heterozygosity per locus. The Miramichi and Gaspereau populations had the highest proportion of polymorphic loci (0.22, mean for the Great Lakes 0.16), and the Miramichi population had the highest mean number of alleles per locus (1.37, mean for the Great Lakes 1.15). The Gaspereau population had the highest average heterozygosity per locus (0.044); however, the mean average heterozygosity for the three Maritime populations (0.034) and the eight Great Lakes populations (0.030) did not differ significantly (P = 0.24). The Cayuga sample was intermediate to the Maritime and the Great Lakes for these diversity parameters with proportion of polymorphic loci = 0.19, mean number of alleles per locus = 1.22, and average heterozygosity per locus = 0.029. The three diversity parameters were not significantly different between the eight samples from the Great Lakes.

Lakes Ontario and Huron were sampled at more than one site. Within these two lakes, no significant difference in allelic frequencies was found between the populations (Ontario: chi-square = 9.62, 15 df, P = 0.84; Huron: chi-square = 2.21, 6 df, P = 0.90). At the Pickering NGS where impinged and unimpinged alewife from the same site were compared, also no significant allelic frequency differences were observed (chi-square = 1.62, 4 df, P = 0.80); thus, these two samples were pooled. The allelic frequencies did not differ significantly (chi-square = 37.1, 49 df, P = 0.89) between the four Great Lakes. The Cayuga and Great Lakes populations resembled each other genetically and shared all but three of their electrophoretic alleles. Two very rare alleles, *mIDHP*150* and *GPI*170*, were found only in the Great Lakes and not in Cayuga Lake (Table 1). Fisher's exact test revealed that the frequencies for these two polymorphisms between the two populations were not significantly different. The allele *PGM-3*93* was found in Cayuga Lake but not in the Great Lakes, and the frequency difference was significant (P = 0.017). For the remaining four polymorphic loci between these two populations, only *LDH-2** had a significant allelic frequency difference, but the cumulative chi-square over all seven polymorphic loci was significant (P = 0.040). In contrast, the Maritime populations were

TABLE 1. Allelic frequencies for alewife from the Great Lakes, Cayuga Lake, and the Canadian Maritimes. Only frequencies for the less common allele (i.e. other than the 100 allele) at each locus are given. The number of individuals sampled from each population is given in parentheses. Frequencies of rare alleles: Grimsby *mIDHP*150*, 0.007; Miramichi River *AK*53*, 0.005; *LDH-1*-468*, 0.005; *mMEP*97*, 0.010; *MPI*92*, 0.025; Gaspereau River *SOD*76*, 0.020; Cayuga Lake *PGM-3*93*, 0.010.

	Locus							
Population	*AK*145*	*CK-2*138*	*GPI*170*	*IDDH*125*	*sIDHP**	*LDH-2*79,87*[b]	*MPI*121*	*PGDH*90*
Lake Ontario								
Cobourg (70)	0.000	—[a]	0.000	0.193	0.000	0.014	0.357	0.000
Pickering (135)	0.000	0.000	0.007	0.196	0.000	0.015	0.313	0.000
Grimsby (72)	0.000	—	0.007	0.167	0.000	0.014	0.319	0.000
Queenston (75)	0.000	—	0.020	0.173	0.000	0.013	0.360	0.000
Lake Erie (72)	0.000	0.028	0.007	0.188	0.000	0.000	0.299	0.000
Lake Huron								
Bruce (72)	0.000	—	0.007	0.160	0.000	0.014	0.306	0.000
South Bay (80)	0.000	0.013	0.013	0.194	0.000	0.013	0.306	0.000
Lake Michigan (72)	0.000	0.014	0.007	0.229	0.000	0.014	0.347	0.000
Cayuga Lake (100)	0.000	0.005	0.000	0.180	0.000	0.035	0.270	0.000
Maritimes								
Miramichi River (100)	0.005	0.000	0.000	0.177	0.000	0.000	0.870	0.020
St. John River (74)	0.000	0.000	0.000	—	0.014	0.000	0.649	0.014
Gaspereau River (74)	0.060	0.000	0.000	—	0.053	0.000	0.513	0.053

[a] Locus not scored for this sample.

[b] Frequencies of alleles with similar electrophoretic mobilities combined for brevity of presentation in this table only.

genetically highly differentiated from each other ($P < 0.001$) and from the Great Lakes and Cayuga Lake populations ($P << 0.001$). Sixteen electrophoretic alleles were found that are not shared by the Maritime and Great Lakes or Cayuga Lake populations. The alleles *AK*53, AK*145, IDDH*67, sIDHP*92, LDH-1*-468, sMEP*97, MPI*92, PGDH*90, PGDH*95*, and *SOD*76* were found only in the Maritime populations, and the alleles *CK-2*138, GPI*170, mIDHP*150, LDH-2*79, LDH-2*87*, and *PGM-3*93* were found only in the Great Lakes or Cayuga Lake.

In a nested gene diversity analysis using Wright's F-statistic (Wright 1978), 89.1% of the total genetic variation was within samples, 7.4% among the three regions Maritime, Great Lakes, and Cayuga Lake, 3.5% among the three Maritime samples, and less than 1% among the Great Lakes samples. The variation within the Great Lakes sampled at more than one site (Ontario and Huron) was also less than 1%. If the Cayuga sample is placed in the hierarchical analysis with the Great Lakes samples, rather than in a separate region, the percentages do not change significantly with 7.9% between the Maritime and the Great Lakes and 3.1% within regions, of which only 0.2% is among the Great Lakes and the Cayuga sample.

Similar dendrograms with almost identical branching patterns were obtained with several measures of genetic distance. Nei's standard genetic distance (Nei 1972, 1975) was chosen to illustrate the genetic relationships among the populations (Fig. 2). This distance measure had a high cophenetic correlation (0.922) and can be related to an evolutionary time scale (Nei 1975). The Great Lakes samples were genetically homogeneous and clustered on a single branch in the dendrogram (Fig. 2). The genetic distances among the Great Lakes samples were not significantly different from zero and too small (mean distance = 0.0006) to illustrate on the figure (Fig. 2). The Cayuga Lake sample clustered closely with the Great Lakes samples, but fell on a separate branch. The Maritime populations, although far removed in the dendrogram from the Great Lakes and Cayuga populations, did not cluster together on a single separate branch. The St. John and Gaspereau populations were more closely clustered with the Great Lakes populations than the Miramichi sample which was on a separate branch. The St. John and Gaspereau populations that are closest geographically among the three Maritime samples (Fig. 1) were also most closely associated in the dendrogram (Fig. 2). These branching patterns were the same for all distance measures compared except for Roger's distance for which the Miramichi and St. John populations were connected on a separate branch.

Discussion

The aim of this study was to provide genetic information about alewife pertaining to their colonization routes and to their population structure in the Great Lakes. Firstly, we wanted to look for any evidence of genetic differentiation among local populations within the Great Lakes. This information was relevant to concerns about adverse effects of impingement by electric power generating stations (Rodgers et al. 1989) and overexploitation by introduced salmonines (Stewart and Ibarra 1991) on local populations of alewife. Secondly, we were interested in the historical biogeography of Great Lakes alewife. In particular, we wanted to determine whether the colonization of the Great Lakes by alewife in the last century (Scott and Crossman 1973) was from the Canadian Maritimes via the St. Lawrence River or whether the colonization route was along the Hudson–Mohawk River system via the Erie Barge Canal and the Finger Lakes.

We conclude that of the two invasion routes proposed (Scott and Crossman 1973), the Hudson–Mohawk rivers via the Finger Lakes is the most probable (Fig. 1). Allelic frequencies (reflected in the dendrogram, Fig. 2) of Great Lakes alewife very closely resemble those of Cayuga Lake and were very different from those for three Canadian Maritime populations, and Great Lakes alleles formed a subset of those found in Cayuga Lake, except for two very rare alleles (*GPI*170* and *mIDHP*150*). In contrast, the Maritime and Great Lakes populations do not share a large proportion of their alleles (15 alleles found to be exclusive to either the Maritime or Great Lakes populations). Furthermore, if the Great Lakes populations were derived from the Canadian Maritimes, they would be expected to resemble the Gulf of St. Lawrence population (Miramichi

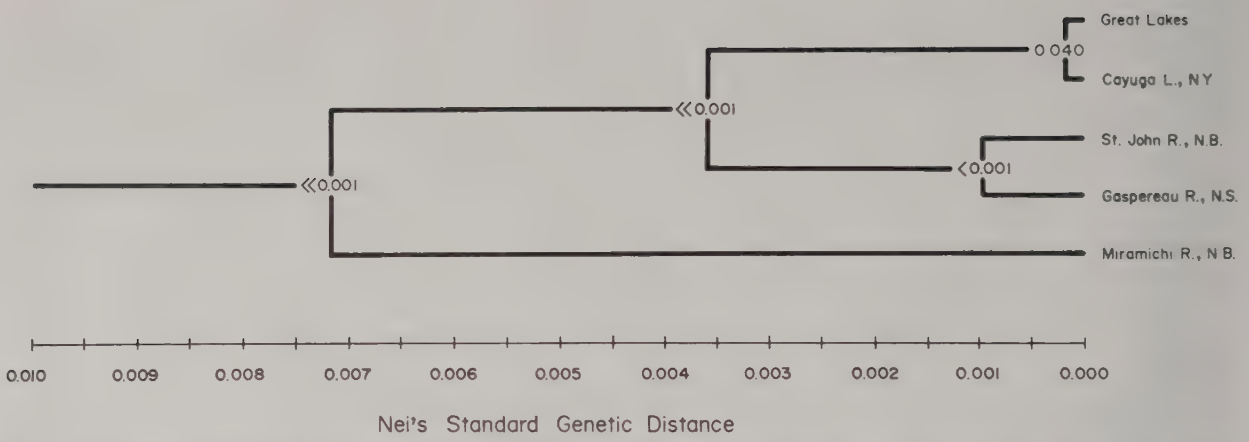

FIG. 2. Dendrogram illustrating the genetic similarity among alewife populations based on Nei's standard genetic distance (Nei 1972) and obtained by UPGMA cluster analysis (Sneath and Sokal 1973). The likelihood ratio chi-square was used to test whether the populations had significantly different allele frequencies. At each branch point in the dendrogram, the probability from the chi-square is given. The seven Great Lakes samples were combined on one branch. The probability of their chi-square was 0.81 and the mean standard genetic distance among these seven samples was 0.0006.

River) more closely than the two Atlantic seaboard populations; however, the reverse is the case (Fig. 2).

The Hudson – Finger Lakes route is similar to that proposed for the invasion of the Great Lakes by the white perch, *Morone americana* (Johnson and Evans 1990), and the sea lamprey, *Petromyzon marinus* (Brussard et al. 1981). The most northerly distribution of anadromous white perch along the North Atlantic coast does not extend to the Gulf of St. Lawrence. It has been postulated that this limit to their distribution is determined by temperature (Johnson and Evans 1990) and prevents them from entering the St. Lawrence River. In contrast, the most northerly distribution of the sea lamprey and alewife extends into the Gulf of St. Lawrence and Newfoundland. Nevertheless, our genetic data indicate that invasion of the alewife did not occur via the St. Lawrence River and that those first observed in the upper St. Lawrence River (Bean 1884; Smith 1892; Toner 1943) probably originated from those that had previously colonized Lake Ontario. Similar to our alewife study, Brussard et al. (1981) found a close genetic affinity, also based on allozyme data, between Lake Ontario sea lamprey and those from the Finger Lakes (Seneca and Cayuga).

It has also been proposed that the origin of Lake Ontario alewife is due to an accidental introduction with a planting of American shad, *Alosa sapidissima*, from the Hudson River in 1870 (Bean 1884). Smith (1892) pointed out that alewife had been observed in Cayuga and Seneca lakes since 1868, prior to the planting of shad in the region. He implied that since there is ''easy communication'' from these lakes with Lake Ontario via the Seneca River, Oswego River,and Oswego Canal, the accidental introduction hypothesis could be rejected. Our genetic data also suggest that the Ontario alewife population did not originate from a single introduction. Such an event would most likely have been accompanied by a population bottleneck resulting in a loss of alleles and change in allelic frequencies relative to the donor population. We did not observe such genetic changes of the Lake Ontario population relative to the Cayuga Lake population.

The genetic homogeneity of alewife from the four Great Lakes, in conjunction with their chronological appearance starting in Lake Ontario and ending in Lakes Michigan and Superior, supports the previously proposed route of invasion of Lake Erie from Lake Ontario and the upper Great Lakes Huron, Michigan, and Superior in succession from the lower Great Lakes. Furthermore, our data indicate that these invasions were not by small numbers of individuals on a single occasion because such bottlenecks would be expected to have resulted in substantial changes in allelic frequencies as well as a reduction in the overall genetic variation. The four Great Lakes populations we examined share the same alleles except for two rare alleles (*mIDHP*150* and *LDH-2*79*) which were found only in one of the Great Lakes each (Table 1). The discontinuous distribution of these two rare alleles is consistent with expected sampling variation for the number of individuals analysed from each lake. Population genetic diversity parameters (average number of alleles per locus, proportion of polymorphic loci, and average heterozygosity per locus) were not significantly different among the Great Lakes populations or between the Great Lakes and Cayuga Lake populations. These results, in conjunction with the allelic frequency data, indicate that neither the initial invasion nor the subsequent colonization of the upper Great Lakes was accompanied by severe population bottlenecks. The data do not rule out the possibility, however, of a continuous leakage of small numbers of invaders. Such continuous immigration, similar to single large-scale invasions, would also maintain genetic parameters the same in the donor and recipient population.

We observed no significant differences in allelic frequencies or genetic diversity parameters either among Great Lakes populations or for samples collected from several spawning sites within Lakes Ontario and Huron. Hence, allozyme data provide no evidence for local stocks of alewife in the Great Lakes. However, allozyme data would not have detected genetic heterogeneity unless local populations had been established by small numbers or if these populations had been genetically isolated for many generations. Genetic drift over the 120 or so years since alewife have been present in the Great Lakes would result in genetic differences much smaller than those that we would have detected. For example, we did not find significant allelic frequency differences corresponding to a Nei standard genetic distance much less than 5×10^{-4} (Fig. 2). However, genetic distances of this order of magnitude correspond to divergence times of thousands of years (Nei 1975). In contrast, for the Maritime populations, which have a much longer zoogeographic history of separation, in the order of 10^4 yr since the last gaciation, substantial genetic differences in allozyme variation were observed.

Even though we did not find differences for allozyme variation among Great Lakes alewife, it is still possible that they are differentiated for quantitative genetic characters. Phenotypic differences between populations for quantitative characters controlled by many genes of small effect (polygenes) accumulate at rates orders of magnitude faster than allozyme frequency differences by genetic drift (Lande 1980; Lande and Barrowclough 1987). For example, if selection is stabilizing and the mean fitness of an individual relative to the optimum phenotype in the population is of the order of a few percent, then 10^2–10^3 generations after passing through a bottleneck are required for a population of approximate effective size of 500 to recover typical genetic variation observed in natural populations. For a recent colonizer, the difference between the optimum phenotype and the mean fitness, called the selection load, may be much larger, leading to even shorter times for adaptation and local differentiation. Perhaps the large die-offs of alewife that have been observed in the Great Lakes (e.g. O'Gorman and Schneider 1986) are symptomatic of the selection load on these populations. If one assumes that the load is 10 times higher for a formerly anadromous invader such as the alewife, about 100 generations are still required for the colonizing populations to reach new adaptive peaks. Since alewife have been present in the Great Lakes for only about 40 generations, they are expected to be still well below their adaptive peaks. Other modes of selection such as directional selection for single traits or disruptive selection are thought to play a lesser role in natural populations, although it has been shown that directional selection in animal and plant breeding can result in substantial changes in quantitative characters in a few generations well within the time that alewife have been present in the Great Lakes. Hence, it is possible that some adaptation to local conditions has occurred but that insufficient time has elapsed for the alewife to have reached optimum fitness in their new habitats.

The degree of genetic differentiation reported for other fishes that have recently colonized the Great Lakes varies considerably. Similar to the alewife, pink salmon, *Oncorhynchus gorbuscha*, were genetically uniform for allozyme variation throughout the Great Lakes, although they differed significantly from anadromous populations of their supposed origin (Gharrett and Thomason 1987). Among sympatric stocks of rainbow smelt, *Osmerus mordax*, in Lake Superior, Schreiner et al. (1984) reported significant differences in allelic frequencies at the serum transferrin (*TF*) locus which were consistent with differences in length–frequency distributions, fecundities, and growth rates (Luey and Adelman 1984). In contrast, MacCrimmon et al. (1983) did not observe genetic differences among rainbow smelt in Lake Erie, in spite of significant differences in growth rates between basins. These apparently contradictory findings may be resolved if one compares the loci analysed for genetic differentiation in these two studies. The Schreiner et al. (1984) study included the *TF* locus, which accounted for most of the genetic differentiation observed. Variation at this locus has been implicated in fitness differences among genotypes (Reinitz 1977; Suzumoto et al. 1977), and, hence, may be subject to directional selection. The MacCrimmon et al. (1983) study, on the other hand, similar to our study on alewife, examined only loci which are generally thought to be more temporally stable. Krueger and May (1987a, 1987b) observed significant differences in allelic frequencies for naturalized brown trout, *Salmo trutta*, and rainbow trout, *Oncorhynchus mykiss*, from rivers draining into Lake Superior, although the level of population differentiation was less than that reported for native populations. These genetic differences in a more *K*-selected salmonid as opposed to a more *r*-selected clupeid such as the alewife (MacLean and Evans 1981) may be due to founder effects, i.e. colonization by small numbers of individuals with different allelic frequencies due to sampling variation. Subsequently, such differences can be maintained and increased by drift because of genetic isolation due to natal homing in salmonids, as suggested by Krueger and May (1987a). Alewife, in contrast, may invade local areas in larger numbers and may not home with the fidelity of salmonids.

At Pickering NGS, impinged alewife are generally similar to nonimpinged alewife, both for allozyme variation and in morphology (Rodgers et al. 1989). The impinged fish, however, weighed significantly less at a given length and had significantly reduced relative liver weights (Rodgers et al. 1989). We do not interpret these differences as evidence of genetic effects, since they would be expected in fish exhausted from swimming for a prolonged period at velocities greater than normal cruising speed, with limited opportunities for feeding (Beamish 1978), conditions which would apply to alewife impinged in the Pickering intake forebay. These differences in condition are also consistent with reports of significant increases in lactic acid levels in the blood of alewife impinged at Pickering NGS (Hutcheson 1981). More extensive sampling throughout the year and the examination of characters under quantitative genetic control would be required to determine whether impingement affects local stocks of alewife genetically. However, our data on the general genetic uniformity of Lake Ontario alewife suggest that impingement will not significantly alter the genetic composition of Great Lakes alewife. Heavily impacted local areas would be readily recolonized by alewife from adjacent populations.

In our samples, Maritime alewife were genetically distinct among collection sites (Fig. 2). The two populations, Gaspereau and St. John rivers, that are in closest geographic proximity and are also less isolated from each other by intervening land masses (Fig. 1) are also genetically most closely related to each other (Fig. 2). This correlation between geographic distance or isolation and genetic distance has been generally observed in allozyme studies (e.g. Ståhl 1987; Ihssen et al. 1988; Utter et al. 1989). The contrast in stock structure between Maritime and Great Lakes alewife likely results, at least in part, from differences in their life histories. The anadromous Maritime alewife return to their natal rivers to spawn (Thunberg 1971; Messieh 1977) and have presumably separated into distinct stocks over the thousands of generations since the last glaciation. In contrast, alewife were first reported in Lake Ontario in the 1870's and have invaded the other Great Lakes only within the last 60 yr. Further, alewife in the Great Lakes spawn on shallow, nearshore beaches and homing has not been demonstrated (Scott and Crossman 1973). The implication is that, even in the longer term, Great Lakes alewife may not develop the stock structure that has been observed for anadromous species with high homing fidelity.

In an earlier study of Maritime alewife, McKenzie (1973) was unable to distinguish electrophoretic patterns for *LDH* or muscle myogen among alewife from three locations on the St. John River and Coventry Bay, Prince Edward Island. These negative results may simply be due to the choice of loci and the resolving power of the techniques employed in early electrophoresis studies. We also found no differentiation at Maritime *LDH* loci in our samples, except for a very rare allele (*LDH-1*-468*) in the Miramichi River. Our results indicate, however, that genetic variation at loci such as *sIDHP**, *AK**, *MPI**, and *PGDH** and possibly *IDDH** and *SOD** will be very useful material for studying differentiation and genetic isolation of Maritime alewife populations.

The overall genetic diversity we observed in Maritime alewife is larger than what has been found for other anadromous clupeid fishes. For example, Shoubridge (1978) found only one polymorphic locus out of 16 loci that he could adequately resolve from four Atlantic populations of American shad ranging from Florida to Quebec. We found 22% (6 out of 27) loci polymorphic in a much more restricted geographic area in the alewife. Bentzen et al. (1989) found low mtDNA sequence divergence (0.2%) in shad for samples collected along the North Atlantic coast from Florida to Quebec. For the Atlantic herring, *Clupea harengus*, Ryman et al. (1984) found only 1% of the genetic variation among 17 locations from the Baltic Sea and Atlantic Ocean, compared with our estimate of 3.5% among the three Canadian Maritime populations of alewife. If we include the Great Lakes – Cayuga Lake populations, which we postulate recently originated from anadromous Atlantic populations and perhaps still resemble these populations, this parameter increases to 12%. The genetic distances between populations in the Ryman et al. (1984) study were typically about 0.001 compared with our mean distance of 0.003. They postulated that either these populations have only diverged recently or that there is substantial gene flow among them. Similarly, Grand (1986) also found a low value of 0.001 for genetic distances of Atlantic herring along the North American coast. In general, the genetic differentiation we observed among the three Maritime populations and the Great Lakes – Cayuga Lake populations is larger than the differentiation that has been previously reported for other marine species (mean for seven species 4.2%, Gyllensten 1985) and similar to anadromous salmonids (mean for four species 8.5%, Gyllensten 1985). This result is a strong indication that Maritime alewife, similar to salmonids, home very precisely to their natal rivers.

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Effect of Female Size and Propagation Methods on Larval Production at a South Carolina Striped Bass (*Morone saxatilis*) Hatchery[1]

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Larval production as a function of female size and hatchery propagation methods was examined for a South Carolina striped bass (*Morone saxatilis*) hatchery from 1988 to 1990. Female weight was not related to egg size or embryo survival among years or between source rivers of females. However, female weight was strongly related to number of hatched embryos due to its collinearity with fecundity. In 1988 and 1990, the offspring of 14 females contributed to 50% of hatchery production. In 1989, only six females contributed to 50% of larval production. In 1990, changes in propagation methods associated with improved female response to hormone injection resulted in more consistent rates of larval production among broods. While the deleterious effects of inbreeding on striped bass populations remain unknown, results indicate that extensive use of large females by hatcheries in fishery recovery programs could decrease effective population size.

Nous avons examiné de 1988 à 1990 la production de larves en fonction de la taille des femelles et des méthodes de propagation utilisées dans une écloserie de bar d'Amérique (*Morone saxatilis*) de Caroline du Sud. Le poids des femelles n'était pas relié à la taille des oeufs ni à la survie des embryons d'une année à l'autre ni selon le cours d'eau d'origine des femelles. Toutefois, le poids était fortement corrélé au nombre d'embryons éclos du fait de son lien colinéaire avec la fécondité. En 1988 et 1990, la progéniture de 14 femelles constituait 50 % de la production de l'écloserie. En 1989, six femelles seulement ont été responsables de 50 % de la production de larves. En 1990, des changements dans les méthodes de propagation, associés à une amélioration de la réponse des femelles à l'injection d'hormones, a permis d'obtenir des taux de production de larves plus réguliers entre les couvées. On ne connaît par les effets néfastes de la consanguinité sur les populations de bar d'Amérique, mais les résultats indiquent que l'emploi à grande échelle de femelles de grande taille dans les écloseries pour les programmes de rétablissement de la pêche pourrait faire baisser la taille effective des populations.

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Over the past decade, artificial propagation techniques have been used to supplement recruitment in declining striped bass (*Morone saxatilis*) fisheries. If hatchery techniques substantially increase early rates of survival and growth of embryos, larvae, and juveniles over those expected under natural conditions, then stocking programs can compensate for lost nursery habitat, spawning biomass, or environmental or anthropogenic perturbations.

The Moncks Corner (South Carolina) Hatchery was the first to develop and apply mass-propagation techniques to striped bass (Harrell 1984; Stevens 1984; Whitehurst and Stevens 1990). During each of its operating years, hundreds of large adult striped bass were collected with electroshocking equipment, principally from the Cooper River but also from the Santee River (Fig. 1). Their offspring were stocked into lakes and reservoirs throughout the United States. In 1986, the Jack D. Bayless Research Fisheries Hatchery (Bayless Hatchery) replaced the Moncks Corner Hatchery in compensation for a large water diversion project. Concurrent with hatchery relocation was a decline in fry (larvae) production (Fig. 2). The production decline was also coincident with a decline in the size and number of brood females available to the hatchery.

Since the early 1980s, serious declines in recruitment (White and Lamprecht 1989) required that stocking into the Santee–Cooper fishery be used as a method for augmenting natural recruitment. Similar or greater numbers of juveniles are stocked as those which naturally recruit (White 1989), and hatchery contribution rates to year-class strengths have exceeded 80% since 1989 (M. White, South Carolina Department of Wildlife and Marine Resources (SCDWMR), Bonneau, SC, pers. comm.). Hence, there is concern about the conservation of the

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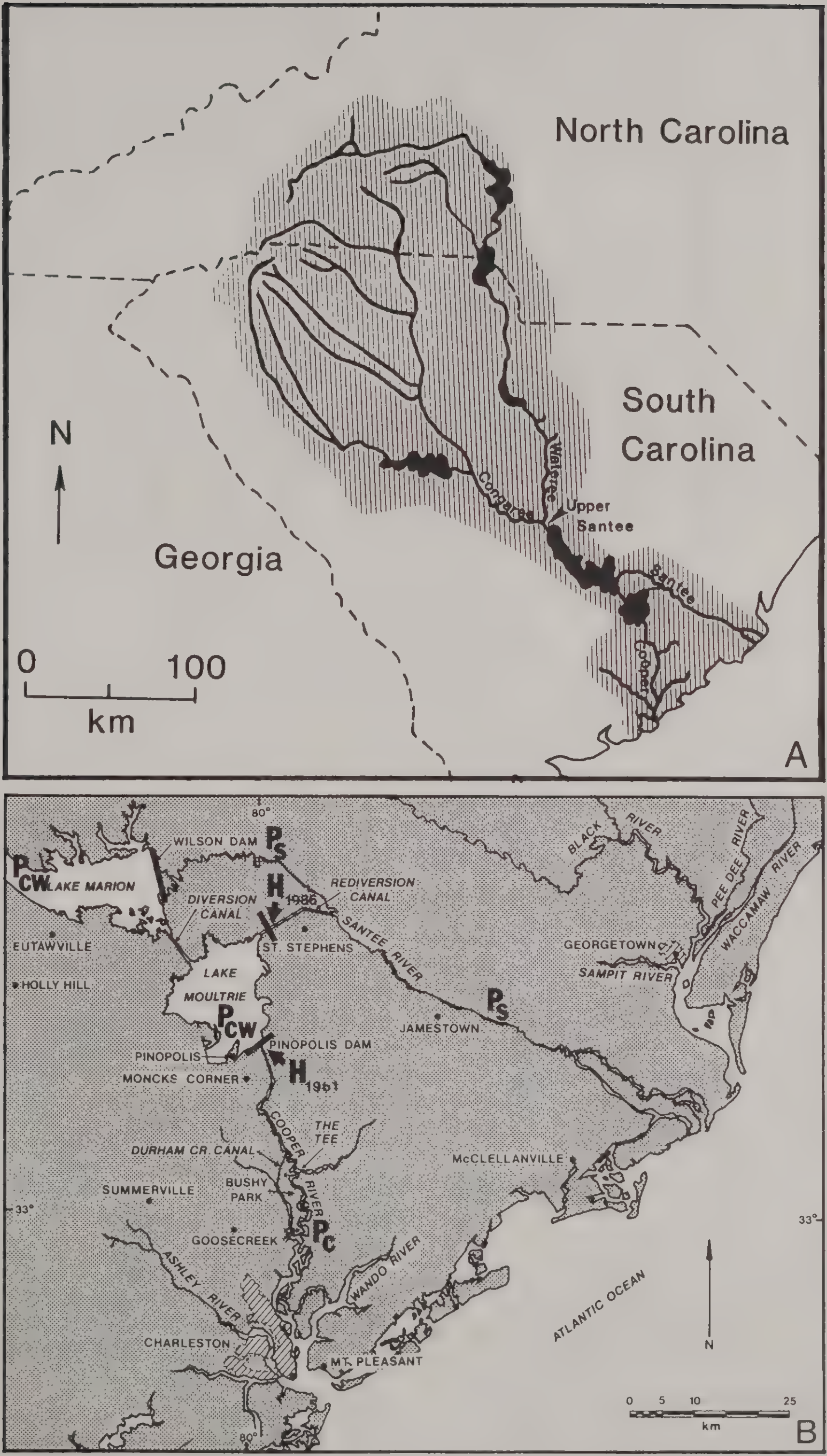
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FIG. 1. (A) Santee River drainage basin. Rivers in which striped bass spawn are labeled. (B) Location of Santee–Cooper ''subpopulations'' and hatcheries within the lower Santee–Cooper system. Note that the Moncks Corner Hatchery (H1961) and the Bayless Hatchery (H1986) are located adjacent to the St. Stephens Dam and the Pinapolis Dam (indicated by bars), respectively. Juveniles are typically stocked into lower Lake Moultrie but in 1990 were also stocked into upper Lake Marion. P_c = Cooper River subpopulation; P_s = Santee River subpopulation; P_{cw} = Congaree/Wateree River subpopulation. Maps adapted from Kjerve (1989).

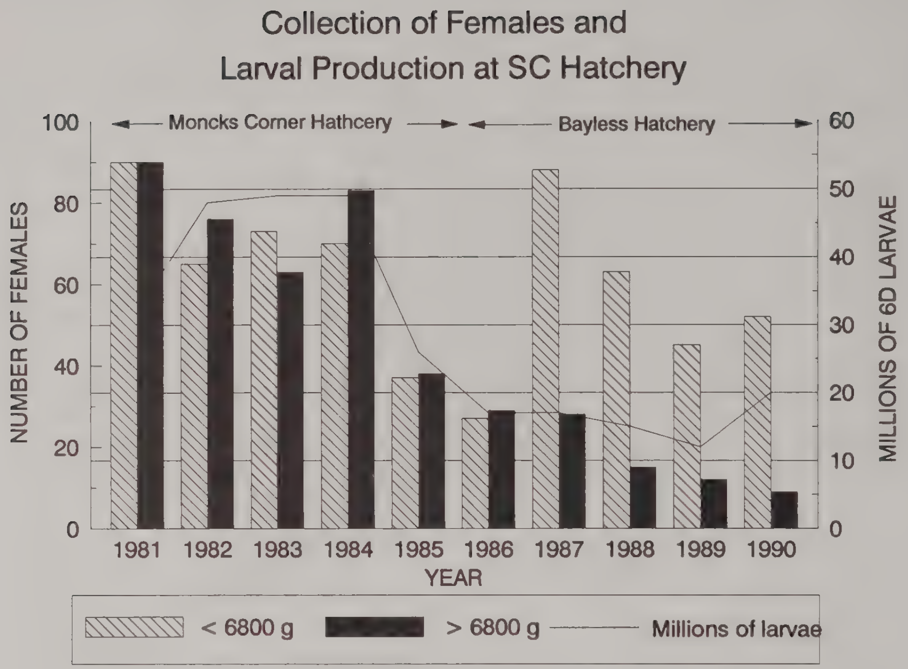

FIG. 2. Decline in brood female size and numbers and reduction of larvae since 1981. In 1984, rediversion began of the Santee–Cooper system's flow into the Santee River. In 1986, hatchery location changed from the Pinopolis Dam to the St. Stephens Dam.

genetic diversity in the Santee–Cooper population, especially during a period when declining numbers and sizes of females were available to the South Carolina hatchery.

Recent hatchery practices in South Carolina and elsewhere are to select the largest females available for propagation purposes (Kerby and Harrell 1990). This strategy is based on the assumption that these fish contribute the most and the highest quality eggs. Fecundity increases linearly with female weight (Lewis and Bonner 1966), and recent studies on Chesapeake Bay striped bass indicate that female size is positively related to egg size, quality, and viability (Zastrow et al. 1989; Monteleone and Houde 1990). However, hatchery propagation methods could affect fecundity and egg size relationships, and the advantage of using only large females at hatcheries has not been evaluated.

Our purpose was to investigate the influence of female attributes and hatchery production methods on striped bass embryo survival and hatchery output. This was studied during a 3-yr survey of practices and performance measures at the Bayless Hatchery. Questions considered included (1) how does female size influence hatchery production?, (2) how many brood females are successfully spawned, and effectively contribute to hatchery production?, (3) what are the relationships between artificial propagation techniques and survival of embryos and larvae?, and (4) what are the implications of using large females on genetic representation of offspring (effective population size). Analyses addressing these questions included size/fecundity relationships, the consequences of egg size on larval production, and among-brood variation in larval production (family size).

Methods

Hatchery Survey

Hatchery methods are described in detail in Bayless (1972) and Rees and Harrell (1990), and a description of specific hatchery operations over the period of the hatchery survey (1988–90) is given in Secor (1990). Females were collected by electrofishing in either the Cooper River or the Santee River. Fish were transported similar distances (in 1988 and 1989) from these rivers. In 1988 and 1989, it was apparent that Cooper River females were not producing as many larvae as Santee River females. Therefore, only Santee River broodstock were collected in 1990. In that year, a rediversion canal flowing into the Santee River and in greater proximity to the hatchery, was used as an additional source of Santee River broodstock (Fig. 1). Females were injected with 275–300 IU of human chorionic gonadotropin per kilogram of body weight to promote vitellogenesis and egg ripening. In 1990, hatchery well water (17°C) was heated to 19°C to increase the effectiveness of the hormone (J. Van Tassel, Department of Natural Resources, Maryland, P.O. Box 1136, Prince Frederick, MD 20678, pers. comm.).

Hatchery Operations and Female Measures

Data were collected on each brood female (Table 1). Measures related to hatchery operations were year (YEAR), site (RIVER), and collection date (DATE) of gravid females and response time to HCG injection (INJ). Measures related to the female were weight of the gravid female (WT), total weight of eggs stripped from the female (prior to fertilization and acti-

TABLE 1. Classification and abbreviations of hatchery measures and estimates.

Hatchery operations

YEAR: year (1988, 1989, 1990)
RIVER: river source for brood females (Santee or Cooper rivers)
DATE or D: day of the year when female collected
INJ or I: injection response (hours from HCG injection to ovulation)

Brood female

WT or W: total weight of female (kg)
ROE or R: total weight of stripped eggs (g)
EGGNO or E: total estimated number of stripped eggs

Egg size

WETEGG: wet egg weight (mg; used to estimate EGGNO)
ADJEGG: wet egg weight adjusted for water hardening (mg)
CHOR: chorion diameter (mm; used to adjust WETEGG)
YOLK or Y: yolk diameter (mm)
OIL or O: oil globule diameter (mm)
EGGWT or Ew: dry egg weight (μg)

Performance

FERT: fertilization rate estimated 3–4 h after stripping
HATCH: hatching rate estimated 40 h after stripping
SURV: overall survival of embryos (estimated as FRYNO/EGGNO)
FRYNO: estimated total number of hatched prolarvae

vation) (ROE), and total number of eggs (EGGNO). Total number of eggs was estimated by dividing total egg weight by individual wet egg weight which was measured from formalin-fixed samples.

Egg Size Measures

Measures related to egg size were oil globule (OIL) and yolk (YOLK) diameters and dry weight (EGGWT). Egg samples from brood females were taken after stripping and prior to fertilization and placed in 5% unbuffered formalin (Bulak et al. 1985). Egg samples could not be obtained from all females, but samples were not biased with respect to year, river, or date (Secor 1990). Twenty eggs were individually measured under a compound microscope at 120 ×. Oil globule, yolk, and chorion perimeters were digitized using a ZIDAS digitizing board and software. For each perimeter, a mean diameter was calculated. For dry weight measures, 30 eggs from each brood were immersed in distilled water for 2 min and placed in preweighed aluminum boats. Eggs were dried at 40°C for 24 h, placed in a vacuum desiccator to cool, and then weighed on an electronic microbalance. To determine individual wet egg weight, 30 eggs were drained on a piece of polyester screen and weighed on an analytical balance. Most egg samples partially hardened following fixation (e.g. chorion diameter was usually greater than egg diameter). To compensate, egg and chorion volumes were calculated using mean diameters. The average difference of these volumes was converted to weight by assuming a density of 1.0, and this difference was subtracted from the average wet egg weight to give adjusted wet egg weight (ADJEGG) for each brood. Precision error averaged 5% or less for all egg measures except for ADJEGG which averaged 10% (Secor 1990).

Performance Measures

Measures of hatchery performance were fertilization rate (FERT), hatching rate (HATCH), embryo survival rate (SURV), and total number of hatched embryos (FRYNO). Fertilization and hatching rates and number of hatched embryos were determined as part of hatchery procedure. Survival rate incorporated all sources of mortality from stripping to hatching and was calculated by dividing FRYNO by EGGNO. An analysis and discussion of precision of performance measures is given in Secor (1990).

Analysis

A subjective classification of production measures was applied to facilitate discussion of correlations (Table 1). To relate hatchery production measures, matrices of simple Pearson correlation coefficients were constructed. Linear regression models were used to relate individual production measures. Performance models were developed using a backward stepwise regression technique. Analysis of covariance (ANCOVA) was used to determine year and river effects on production relationships. Statistical analysis was performed using mainframe SAS and PC Statgraphic statistical software.

Results

River and Year Differences

Over the first 2 yr of the survey, significant differences in production measures occurred between females collected in the Cooper and Santee rivers (Table 2). Santee River fish were slightly smaller but performed much better in terms of response time to injection, fertilization, hatching, and survival rates and average number of hatched embryos. Due to these differences and the proximity of the rediversion canal to the hatchery (see Methods, Fig. 1), only Santee River broodstock was used during the 1990 hatchery season. To eliminate the confounding influence of this shift in hatchery procedures, RIVER effects were only examined for data from 1988 and 1989 and YEAR effects were analyzed for only Santee River brood females. Since YEAR effects were significant for DATE and YOLK (Table 2B) and comparisons of means showed that 1988 and 1989 were similar but significantly less than 1990 measures, 1988 and 1989 data were pooled and compared with 1990. Three Pearson correlation matrices were also constructed: (1) Cooper River broods from 1988 and 1989 (Table 3A), (2) Santee River broods from 1988 and 1989 (Table 3B), and (3) Santee River broods from 1990 (Table 3C). River comparisons are between matrices 1 and 2 and year comparisons are between matrices 2 and 3.

Hatchery Operation Effects

Santee River broods were not as strongly affected by DATE or INJ as Cooper River broods (Table 3A versus 3B). Date was positively correlated with egg size and performance measures for both rivers and indicated improvement of hatchery performance later in the season. Cooper River brood measures EGGNO, OIL, and EGGWT were also significantly related to date, suggesting that Cooper River fish might not be as prepared to ovulate as Santee River fish over the hatchery season. Duration of female response to hormone injection was inversely related to egg size for all years (Table 3A and 3B) and fertilization in 1988 and 1989 (Fig. 3). Cooper River brood performance parameters were more tightly linked to INJ (Table 3A versus 3B), again suggesting that these fish were not as advanced in their reproductive cycle as were Santee River fish.

In 1990, changes in hatchery methods appear to have reduced the effect of hatchery operations on performance (Table 3B ver-

TABLE 2. Comparison of means (*t*-test) between (A) rivers and (B) years. River comparisons were made for only 1988 and 1989; year comparisons were made for only Santee River broods. *significant difference in comparison at $P = <0.05$; SD = standard deviation. For hatchery abbreviations and units of measurements on hatchery estimates, see Table 1.

A. River comparisons for 1988 and 1989 data

	Santee River		Cooper River			
Measure	$\bar{X}$ (SD)	N	$\bar{X}$ (SD)	N	t	P
DATE	106 (10)	42	109 (12)	62	−1.3	0.2
INJ*	38.5 (5.9)	42	41.9 (5.9)	62	−2.9	0.004
WT*	5642 (2045)	39	6376 (1441)	59	−2.1	0.04
ROE	746 (520)	40	833 (347)	56	−1.0	0.3
EGGNO × 1000	826 (535)	34	1010 (433)	45	−1.7	0.09
YOLK	1.03 (0.07)	37	1.03 (0.08)	48	0.3	0.7
OIL	0.61 (0.047)	37	0.61 (0.05)	48	−0.3	0.7
EGGWT	256 (40)	37	259 (43)	48	−0.4	0.7
FERT*	0.43 (0.23)	42	0.32 (0.22)	60	2.4	0.02
HATCH*	0.84 (0.28)	41	0.71 (0.16)	45	2.0	0.05
SURV*	0.30 (0.25)	34	0.17 (0.16)	45	2.7	0.009
FRYNO × 1000*	290 (344)	42	175 (174)	62	2.2	0.03

B. River comparisons for Santee River data

	1988–89		1990			
Measure	$\bar{X}$ (SD)	N	$\bar{X}$ (SD)	N	t	P
DATE*	106 (10)	42	100 (12)	61	2.9	0.00 4
INJ	38.5 (5.9)	42	40.1 (7.0)	61	−1.3	0.2
WEIGHT	5641 (2045)	39	5634 (1479)	61	0.02	1.0
ROE	746 (520)	40	831 (346)	60	−1.0	0.3
EGGNO × 1000	826 (535)	34	1026 (520)	43	−1.7	0.1
YOLK*	1.03 (0.07)	37	1.09 (0.05)	44	−4.1	0.0001
OIL	0.61 (0.047)	37	0.63 (0.03)	44	−1.4	0.2
EGGWT	256 (40)	37	249 (33)	44	−0.8	0.4
FERT	0.43 (0.23)	42	0.42 (0.22)	59	0.4	0.7
HATCH	0.84 (0.28)	41	0.80 (0.31)	61	0.5	0.6
SURV	0.30 (0.25)	34	0.36 (0.20)	43	−1.2	0.2
FRYNO × 1000*	290 (344)	42	322 (251)	61	−0.5	0.6

sus 3C). However, significant negative correlations did occur between reproductive output (ROE and EGGNO) and DATE; the weight and number of eggs decreased as the season progressed. Most noteworthy is the absence of INJ effects on all performance measures (Table 3C).

Female Effects

Female weight was highly correlated with ROE and EGGNO across rivers and years. The relationship of egg number to female weight was not affected by year (ANCOVA; Cooper River data excluded: $N = 76$; $F = 2.57$; $P = 0.083$) or river (ANCOVA; 1990 data excluded: $N = 78$; $F = 0.10$; $P = 0.75$). Fecundity for mature females was estimated at 230 000 eggs/kg (Fig. 4) which is consistent with published reports of Atlantic Coast striped bass populations (Setzler-Hamilton et al. 1980). FRYNO was also highly correlated with female weight; correlations were stronger for Santee River broods than for Cooper River broods (Fig. 5). Female weight was not related to egg size among years or between rivers (Table 3). Santee River data for 1988–89 showed significant correlation between female weight and egg survival (Table 3B), but as shown below, this was probably due to colinearity of female weight with ROE where ROE explained most of the variation in egg survival (see Best Model for Hatchery Production).

Egg Size Effects

The relation of egg size to performance varied dramatically between rivers and years. Most egg size measures were correlated with performance during 1988 and 1989, but no correlations occurred for 1990 (Table 3; Fig. 6). This may be the result of less variance in egg size and performance measures for 1990 (Table 2B). Peformance for Cooper River broods was related to all measures of egg size in contrast with performance for Santee River broods which was only related to yolk diameter and dry egg weight.

Best Model for Hatchery Production

Because many measures might be collinear in their effects, a stepwise regression analysis was used to model performance measures for each data group. Significant differences in models occurred between rivers and years (Table 4). In the Santee River (1988, 1989), performance was related to INJ and reproductive output measures (Table 4A). Because female weight was not selected in this procedure, its effects on fertilization rate (Table 3A) must be collinear with reproductive output measures. Cooper River performance was almost exclusively related to INJ (Table 4B). In 1990, only FRYNO showed significant correlation with surveyed measures; SURV, HATCH, and FERT were all independent of hatchery operations, female, and egg size effects (Table 4C).

TABLE 3. Pearson correlation matrices for various measures and estimates. (A) 1988–89, Cooper River data; (B) 1988–89, Santee River data; (C) 1990, Santee River data. Coefficients are only listed if significant at $P < 0.05$; otherwise, a dash is listed. For abbreviations and units of measurements on hatchery estimates, see Table 1.

	D	I	W	R	E	Y	O	Ew
	A. 1988–89, Cooper River							
Hatchery operations								
DATE	1							
INJ	−0.50	1						
Female effects								
WT	—	—	1					
ROE	—	—	0.69	1				
EGGNO	−0.28	—	0.58	0.89	1			
Egg size								
YOLK	0.73	−0.53	—	—	—	1		
OIL	0.55	−0.49	—	—	—	0.76	1	
EGGWT	0.37	−0.61	—	0.37	—	0.77	0.69	1
Performance								
FERT	0.46	−0.60	—	—	—	0.54	0.39	0.61
HATCH	—	−0.52	—	—	—	0.35	0.32	0.50
SURV	0.38	−0.61	—	—	—	0.49	0.38	0.50
FRYNO	—	−0.43	0.29	0.50	0.38	—	—	0.39
	B. 1988–1989: Santee River							
Hatchery operations								
DATE	1							
INJ	−0.52	1						
Female effects								
WT	—	—	1					
ROE	—	—	0.92	1				
EGGNO	—	—	0.88	0.94	1			
Egg size								
YOLK	0.55	−0.66	—	—	—	1		
OIL	—	−0.43	—	—	—	0.70	1	
EGGWT	—	−0.49	—	—	—	0.63	0.68	1
Performance								
FERT	0.43	−0.64	0.51	0.56	—	0.51	—	0.33
HATCH	—	−0.46	—	—	—	0.35	—	0.34
SURV	0.48	—	0.40	0.44	0.36	0.47	—	—
FRYNO	—	—	0.84	0.89	0.38	0.21	—	—
	C. 1990, Santee River							
Hatchery operations								
DATE	1							
INJ	−0.44	1						
Female effects								
WT	—	—	1					
ROE	−0.30	—	0.85	1				
EGGNO	−0.32	—	0.82	0.90	1			
Egg size								
YOLK	0.38	−0.41	—	—	—	1		
OIL	—	—	—	—	—	0.50	1	
EGGWT	—	−0.40	—	—	—	0.80	0.71	1
Performance								
FERT	—	—	—	—	—	—	—	—
HATCH	—	—	—	—	—	—	—	—
SURV	—	—	—	—	—	—	—	—
FRYNO	—	—	0.55	0.58	0.61	—	—	—

Variance in Hatchery Peformance: Year, River, and Brood

The number of hatched embryos per female was significantly greater for Santee River broods than for Cooper River broods (Table 2). Performance was much more strongly related to female weight in the Santee River than in the Cooper River (Fig. 5). The plot for the Santee River showed that most of the production came from the eight largest females.

To examine yearly variation in production rates among broods, females were ranked in ascending order of performance and plotted against cumulative number of fry for each year. River data were combined for this analysis (Fig. 7). Note that for all years, the top-ranked female produced about 10% of the entire year's production. In 1988 and 1990, 14 females contributed to 50% and 40 females contributed to 90% of hatchery

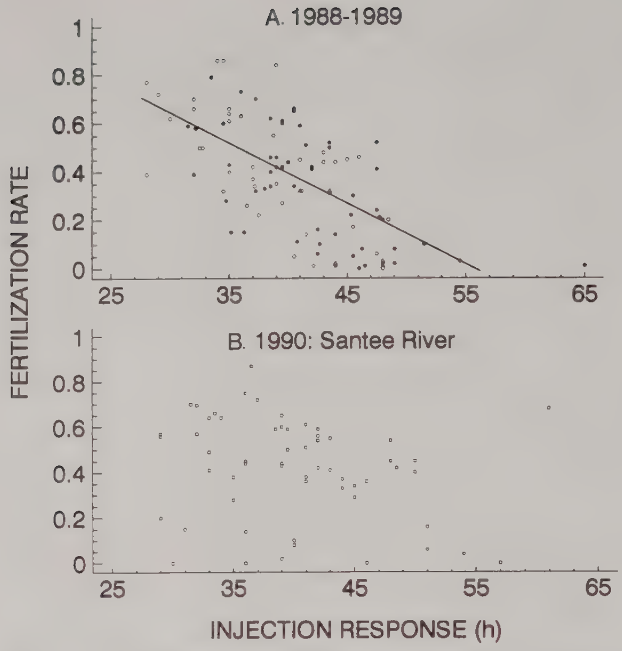

FIG. 3. Injection response versus fertilization rate for (A) 1988–89 data and (B) 1990 data. Cooper and Santee rivers are shown by solid and open symbols, respectively. (A) 1988–89 (regression from pooled Santee and Cooper River data): FERT (%) = 1.328 − 0.0239 · INJ (h); $N = 111$; $F = 56$; $P = 0.0001$; $R^2 = 0.34$. (B) 1990: FERT (%) = 0.710 − 0.000729 · INJ (h); $N = 59$; $F = 3.3$; $P = 0.07$; $R^2 = 0.05$. 1990 (outlier removed): FERT (%) = 0.777 − 0.00955 · INS; $N = 58$; $F = 40$; $P = 0.05$; $R^2 = 0.068$.

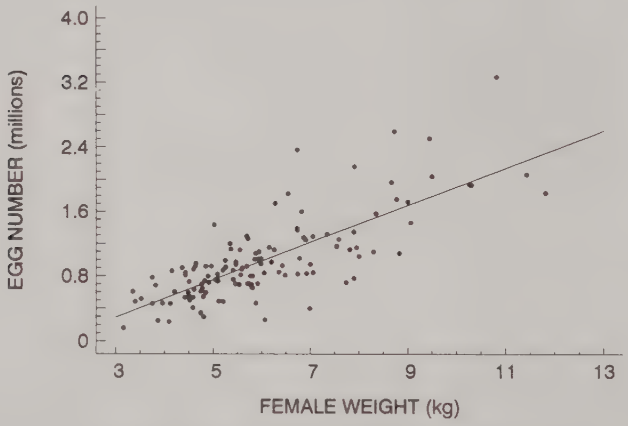

FIG. 4. Female weight versus number of eggs stripped. EGGNO = −400,193 + 230 722 · WT (g); $N = 129$; $F = 185$; $P = 0.0001$; $R^2 = 0.59$.

production. In contrast, the 1989 season produced 50% of its production from only six females and 90% of its production from 16 females.

Embryo survival rates averaged from 17 to 30% between rivers and from 22 to 36% among years. This is approximately twofold higher than embryo survival rates reported in Virginia rivers (Olney et al. 1991).

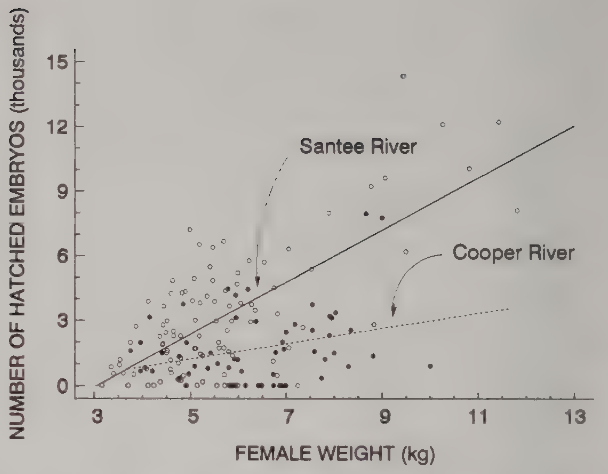

FIG. 5. Female weight versus number of hatched embryos for 1988–89 data. Santee River (open symbols): FRYNO = −51 535 + 144 487 · WT (g); $N = 39$; $F = 91$; $P = 0.0001$; $R^2 = 0.71$. Cooper River (solid symbols): FRYNO = −55 694 + 35 365 · WT (g); $N = 59$; $F = 5.5$; $P = 0.02$; $R^2 = 0.09$.

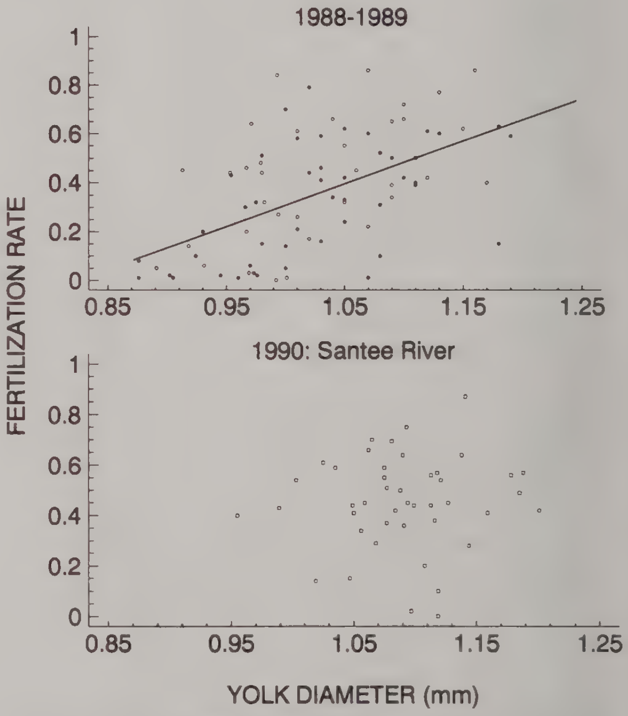

FIG. 6. Mean yolk diameter versus fertilization rate for (A) 1988–89 data and (B) 1990 data. Cooper and Santee Rivers are shown by solid and open symbols, respectively. (A) 1988–89 (regression from pooled Santee and Cooper River data): FERT (%) = −1.399 + 1.713 · YOLK (mm); $N = 91$; $F = 28.5$; $P = 0.0001$; $R^2 = 0.24$. (B) 1990: FERT (%) = 0.205 + 0.227 · YOLK (mm); $N = 44$; $F = 0.2$; $P = 0.7$; $R^2 = 0.00$.

Discussion

The hatchery currently stocks the Santee–Cooper reservoirs at a rate of 2–3 million juveniles per year. Natural recruitment in the Congaree/Wateree stock was estimated to be between 200 000 and 800 000 juveniles per year in 1983 and 1984 (White 1989). If survival of stocked juveniles is even a small fraction of natural juvenile survival (e.g. 5%), then the hatchery

TABLE 4. Best models for hatchery performance. (A) 1988–89, Santee River; (B) 1988–89, Cooper River; (C) 1990, Santee River. Variable inclusion based on backwards stepwise regression procedure. For hatchery abbreviations and units of measurements on hatchery estimates, see Table 1.

Performance measure	Selected variables	Coefficient	t	P	R^2
A. 1988–89, Santee River					
FERT	Constant	1.070	—	—	—
	INJ	−0.0213	−5.0	0.000	—
	ROE	0.000245	5.1	0.000	0.67
HATCH	Constant	1.711	—	—	—
	INJ	−0.0232	−3.0	0.005	0.20
SURV	Constant	1.197	—	—	—
	INJ	−0.0261	−5.3	0.000	—
	EGGNO	1.490	2.6	0.01	0.52
FRYNO	Constant	5.223	—	—	—
	INJ	−1.865	−5.2	0.000	—
	EGGNO	0.607	14.5	0.000	0.88
B. 1988–89, Cooper River					
FERT	Constant	0.422	—	—	—
	INJ	−0.0144	−2.5	0.02	—
	EGGWT	0.00194	2.3	0.02	0.45
HATCH	Constant	2.119	—	—	—
	INJ	−0.0341	−4.3	0.000	0.29
SURV	Constant	0.852	—	—	—
	INJ	−0.0160	−5.1	0.000	0.36
FRYNO	Constant	5.090	—	—	—
	INJ	−1.311	−3.8	0.000	—
	ROE	261.4	4.2	0.000	0.44
C. 1990, Santee River					
FERT	—	—	—	—	—
HATCH	—	—	—	—	—
SURV	—	—	—	—	—
FRYNO	Constant	1.615	—	—	—
	ROE	450.2	5.7	0.000	—
	OIL	−2.635	−2.8	0.008	0.46

is making a notable contribution (e.g. 13%) to local stocks. Preliminary studies on poststocking survival based on recent collections by SCDWMR of marked juveniles and young adults (Secor et al. 1991) indicate contribution rates which are substantially higher than this hypothetical case (M. White, SCDWMR, Bonneau, SC, pers. comm.).

Broodstock Effects

The Santee–Cooper hatcheries have typically targeted the biggest females in their collections because large females were thought to produce not only greater numbers of eggs but better quality eggs. Results of the survey show that, for the size range tested, female size was related neither to egg size nor egg viability. Female size was correlated with number of fry in all years and egg viability for Santee River in 1988 and 1989 due to its collinearity with number of eggs. Therefore, hatchery production rates have declined as a result of declines in overall numbers of eggs rather than egg quality.

The lack of a positive relationship between female size, egg size, and egg survival was unexpected, given research on other striped bass populations. Rogers and Westin (1981) collected eggs from the Moncks Corner Hatchery and hatcheries on the Hudson River and Chesapeake and found a positive correlation between combined data for egg weights and female lengths. However, their fig. 4 (p. 106) shows no correlations for either the Santee–Cooper or Hudson populations. The significance of the least squares regression from data of the combined populations was entirely due to the Chesapeake population which also showed the greatest range in female length and egg weight. Zastrow et al. (1989) collected eggs from two hatcheries on the Chesapeake and observed strong correlations between egg size and female weight and egg size and embryo survival and weaker correlations between female size and embryo survival. Egg dry weights for the Chesapeake females (170–430 μg) were only slightly greater than those for Santee–Cooper females (130–370 μg). However, over half of the Chesapeake females ranged between 9 and 23 kg whereas fewer than 10% of the Santee–Cooper females ranged above 9 kg and the biggest female weighed 12.2 kg. The lack of correspondence of the survey's results with those of Zastrow et al. (1989) could be due to the differences in range of female weights, differences in populations, or differences in hatchery operations.

Artificial Propagation Effects

Hatchery operations themselves were probably more important in production results than were broodstock effects. For instance, larger Cooper River females did not produce proportionately greater amounts of larvae. Also, greater numbers of larvae were produced in 1990 than in previous years without an increase in female size. Heating hatchery water to promote injection response and using exclusively Santee River females

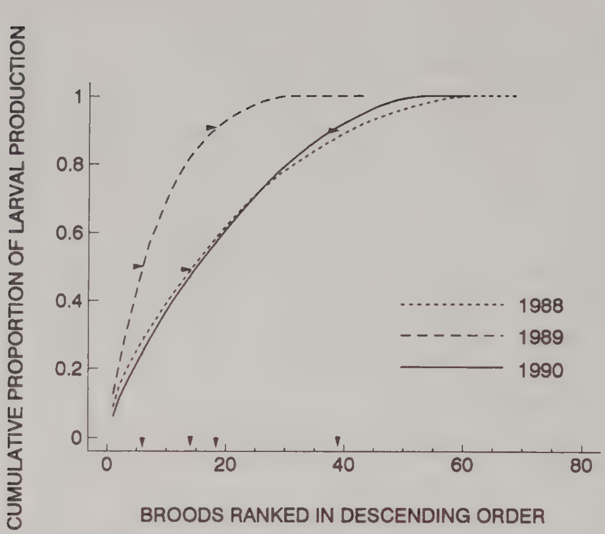

FIG. 7. Ranked broods versus cumulative production rates. Broods are ranked each year by FRYNO in descending order and plotted against percentage of annual production of larvae. Arrows indicate 50 and 90% larval production rates (see text).

in 1990 significantly reduced the effects of hatchery operations (DATE and INJ) and simultaneously increased performance.

An alternative explanation for the reduced effect of hatchery operations in 1990 is decreased transport time of the females due to greater proximity of the hatchery to the collection site. Typical transport time (time in hauling tank) was between 30 and 60 min in 1988 and 1989 and between 5 and 10 min in 1990. In 1988–89, a 1900-L hauling tank with oxygenated water was used and no immediate mortality was observed. Occasionally, females were held for periods of up to 3 h with no mortality observed. In 1990, single females were hauled in 300 L of water. It is our belief that mortality due to transport is related to handling rather than transport time. Because the number of times individual fish were handled was similar among years, we attribute the lack of hatchery effects in 1990 to the change in temperature at which females were held for ripening at the hatchery.

Temperature effects on larval production can be indirectly related to date and river source of broodstock. The Bayless Hatchery starts its season as soon as females can be collected to insure that it meets stocking demands. Water temperature varies between 13°C at the beginning and 21°C at the end of the season (F. W. Sessions, pers. obs.). Peak spawning in the Congaree and Wateree rivers typically occurs at a temperature of 20°C (Bulak et al. 1985). If Santee and Cooper females spawn at similar temperatures, then natural spawning should occur during the latter part of the hatchery season. This could explain the positive correlation between date, egg size, and performance. The Cooper River is typically several degrees cooler than the Santee River during April, and therefore, performance was reduced for Cooper River females. The lack of an effect of date on egg size and performance in 1990 was probably due to heating the water in which females were held to 19°C, a temperature close to that occurring during peak spawning in nature. In 1988 and 1989, females were held in hatchery water at 17°C and presumably egg development was retarded (Rees and Harrell 1990).

Eggs spawned in nature may be larger on average than those produced artificially. Oil globules from a sample of field-collected eggs range from 3 to 15% larger than hatchery-collected egg oil globules (Secor 1990). We speculate that hatchery-spawned eggs are smaller because vitellogenesis has been accelerated by artificial means and is therefore incomplete. To some extent, this may be rectified by raising the temperature during final egg ripening, as was done at the Bayless Hatchery in 1990.

Because egg performance was related to hatchery operations, we cannot discount the effect of female size on egg quality in naturally spawning populations. Large females could be very important members of a spawning population due to either increased egg quality or reproductive output (Zastrow et al. 1989; Monteleone and Houde 1990). However, in the hatchery, female size caused increased hatchery output only through increased fecundity and did not improve egg viability. Therefore, a prudent strategy for minimizing the hatchery's impact on the genetic structure of natural populations would be to use many smaller females rather than fewer larger ones.

Hatchery Contribution to Natural Stocks

In 1990, the overwhelming majority of stocked juveniles in upper Lake Marion (a primary stocking site for the Santee–Cooper system) were the offspring of a single female (92%). While this may be a rare occurrence for the Santee–Cooper Recovery Program, other state and federal agencies typically use very few females in their stocking programs (Kerby and Harrell 1990). Clearly, the topic of genetic conservation in recovery programs is relevant to most striped bass populations.

Effective population number (N_e) is used to evaluate the potential for inbreeding in small populations which do not meet the criteria of an ideal population (Falconer 1981; Gall 1987). The probability of inbreeding is inversely related to N_e (Falconer 1981). To contrast genetic representation of hatchery-produced progeny among years, N_e was calculated to correct for unequal family sizes (Crow and Kimura 1970). Under the assumption of an equal male to female ratio, calculated N_e values were 92, 59, and 110 for 1988, 1989, and 1990, respectively. The lower N_e in 1989 could not be completely accounted for by the fewer females used that year. For instance, 28% fewer females were used in 1989 than in 1990, but N_e was 46% less for 1989 than for 1990. Genetic representation of larvae was affected by variance of family sizes which was influenced by female size and hatchery propagation methods. Recommended levels of N_e for broodstock used in fishery recovery programs range from 200 (Kincaid 1976) to 424 (Tave 1986).

The effect of genetic bottlenecks and inbreeding is complex in striped bass, which are iteroparous, long-lived, and have highly variable recruitment rates. Information on the genetic structure of striped bass populations is limited (Chapman 1989, 1990; Wirgin et al. 1989) but suggests that observed low levels of genetic variation are related to migratory and spawning behaviors which periodically restrict N_e (Chapman 1990). Until more is known about the deleterious effects of inbreeding in striped bass populations, a conservative strategy towards increasing N_e in stocking programs would be prudent. This would include selecting female sizes which are representative of their proportion in the natural spawning population (Falconer 1981). In the absence of dominant year classes, this will mean using greater numbers of small females which should result in more consistent production and increased N_e.

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Effects of Discharge and Substrate Stability on Density and Species Composition of Stream Insects

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Effects of discharge/substrate stability relationships on the abundance, diversity, and distribution of aquatic insects were studied from 1986 to 1988 in three reaches of a small stream in the Manitoba escarpment. Substrate stability (percentage of streambed paving material at incipient motion) ranged from 80 to 20% at bankfull discharge. Eleven taxa of mayflies, 15 taxa of caddisflies, and 10 taxa of stoneflies were collected. All but a few taxa were present at all stations. Benthic insect densities were negatively correlated with increasing discharge and substrate particle movement at all stations. Regression models describing insect density in terms of substrate stability usually accounted for more variation than models describing density in terms of discharge. Insect density decreased up to 94% at the most unstable stream reach following summer and autumn spates when 8 and 12%, respectively, of the streambed paving material was at incipient motion wheres no reduction occurred in a stable riffle constructed nearby. Most insect species were highly resilient; densities and diversities returned to prespate levels during periods of low flow. Temporal and spatial aspects of substrate stability should be considered in stream insect studies, particularly in relation to disturbance.

De 1986 à 1988, on a étudié les incidences des relations entre le débit et la stabilité du substrat dans trois tronçons d'un petit cours d'eau de l'escarpement du Manitoba sur l'abondance, la diversité et la répartition des insectes aquatiques. La stabilité du substrat (soit le pourcentage de matériaux recouvrant le lit du cours d'eau en début de mouvement) variait de 80 à 20 % au cours du débit à plein bord. On a recueilli 11 taxons d'éphémères, 15 taxons de phryganes et 10 taxons de perles; tous étaient présents, sauf quelques-uns, à toutes les stations. La densité des insectes benthiques était en corrélation négative avec un plus grand débit et un plus grand déplacement des particules du substrat à toutes les stations. Les modèles de régression décrivant la densité des insectes en termes de la stabilité du substrat tenaient généralement compte d'une plus grande variation que les modèles décrivant la densité en termes de débit. La densité des insectes a montré une baisse allant jusqu'à 94 % dans les tronçons les plus instables après les crues d'été et d'automne, lorsque 8 et 12 % respectivement des matériaux recouvrant le lit du cours d'eau commençaient à se déplacer, tandis qu'aucune baisse du genre n'a été notée dans un radier stable voisin. La plupart des espèces d'insectes se sont facilement adaptées à ces conditions; ainsi, leur densité et leur diversité se sont rapidement rétablies au niveau pré-crue au cours des périodes de faible débit. On devrait donc tenir compte des aspects temporaux et spatiaux de la stabilité du substrat lors d'études sur les insectes peuplant au cours d'eau, en particulier en ce qui a trait aux perturbations.

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Physical disturbance, usually in the form of floods, may be the dominant determinant of community structure in streams (Resh et al. 1988). The negative effects of floods on riverine insect populations are well known (e.g. Hynes 1970; Gray and Fisher 1981; Sagar 1986). However, insect densities in rivers with large, stable substrates may not be reduced by floods (Cowie 1980; Giberson and Hall 1988). Allen (1959) reported that flows affected insect densities differently depending upon the stability of substrate.

Hydrologists routinely analyze substrate stability when designing drainage channels (Chow 1964). However, although substrate stability is a component of aquatic insect habitat (Newbury 1984), it has rarely been quantified in aquatic entomological studies. It is important to note that as water depth increases, so does the tractive force (shear stress) acting on the substrate and any insects associated with it. Also, the substrate stability of a reach of stream is a function of the dominant stream forming material (bed paving material) and the average slope and depth of water.

Rivers with unstable substrates are characterized by reaches with low species diversity (Cobb and Flannagan 1990; Flannagan et al. 1990); those species present often have life cycle or behavioral adaptations characteristic of frequently disturbed environments (Sagar 1986; Scrimgeour and Winterbourn 1989; Cobb et al. 1991a). Experimental disturbances of artificial substrates or patches of substrate have also reduced insect density (Clifford 1982; Reice 1985; Robinson and Minshall 1986; Doeg et al. 1989); however, recovery was rapid and overall community composition was not altered. Considerable insight into recovery of insects on individual stones or patches of substrate was provided in the experiments of Malmqvist and Otto (1987). However, their experimental plots were small, and recovery pathways may have been quite different relative to whole reaches of stream.

The purpose of the present study was to investigate the effects of substrate stability/discharge relationships upon the density and diversity of aquatic insects of a small natural stream, Wilson Creek, Manitoba; such spacial/temporal studies are lacking (Minshall 1988). The hypothesis of this study was that the degree of impact of floods on aquatic insect density and diversity would depend upon the intensity of discharge and the associated substrate stability.

Materials and Methods

Study Area

Wilson Creek, a fourth-order stream (1:50000 scale) with a drainage area of 22 km^2, is located in west-central Manitoba (50°43′N, 99°33′W) (Fig. 1). The watershed was the site of a 25-yr study to investigate control of flooding problems in the surrounding agricultural district (Newbury 1983). A gauging station has been continuously operated for over 30 yr, resulting in a large database of hydrological and meteorological information. Wilson Creek is part of the Dauphin Lake drainage basin; many of the rivers in this basin have been channelized to improve agricultural drainage (Chapman 1987). The result of stream channelization has been substrate instability, sedimentation, and other related problems (Newbury and Gaboury 1988; Cobb and Flannagan 1991).

Three stations were established in different reaches of the stream (Fig. 1; Table 1). Station W1, located 100 m upstream from a gauging station, was the least stable reach; the streambed paving material was composed of shale deposited from upstream eroding reaches. Station B1 was located 3 km upstream from station W1 in a reach of higher gradient; substrate here was composed of large shale, cobble, and small boulders overlying gravel. The shale was transported during moderate floods whereas larger material moved during peak flows; the streambed paving material was more stable at bankfull discharge than at station W1. Station B2, a boulder-dominated channel, was located 3 km upstream of station B1; it was the most stable reach. Another stable site (W1S) was constructed within the most unstable reach of Wilson Creek using local field stone with a median diameter of 10 cm.

Riparian vegetation at all stations consisted primarily of alders; surrounding forest was predominantly spruce, aspen, and birch. Groundwater inputs throughout Wilson Creek resulted in uniform thermal and chemical gradients along the length of the study area. Macrophyte growth within the creek was rare, but a dense coating of marl (calcium carbonate) occurred on rocks during low discharge periods. During floods, the marl was either scoured off or dissolved.

Hydrology and Substrate Stability

The critical tractive force required to move a rounded substrate is approximately equal to the median diameter (centimetres) of the particle (Lane 1955). Approximately one half the critical tractive force is required to move an equal size of shale because of its shape and low density (Magalhaes and Chau 1983); this adjustment was made during estimates of substrate

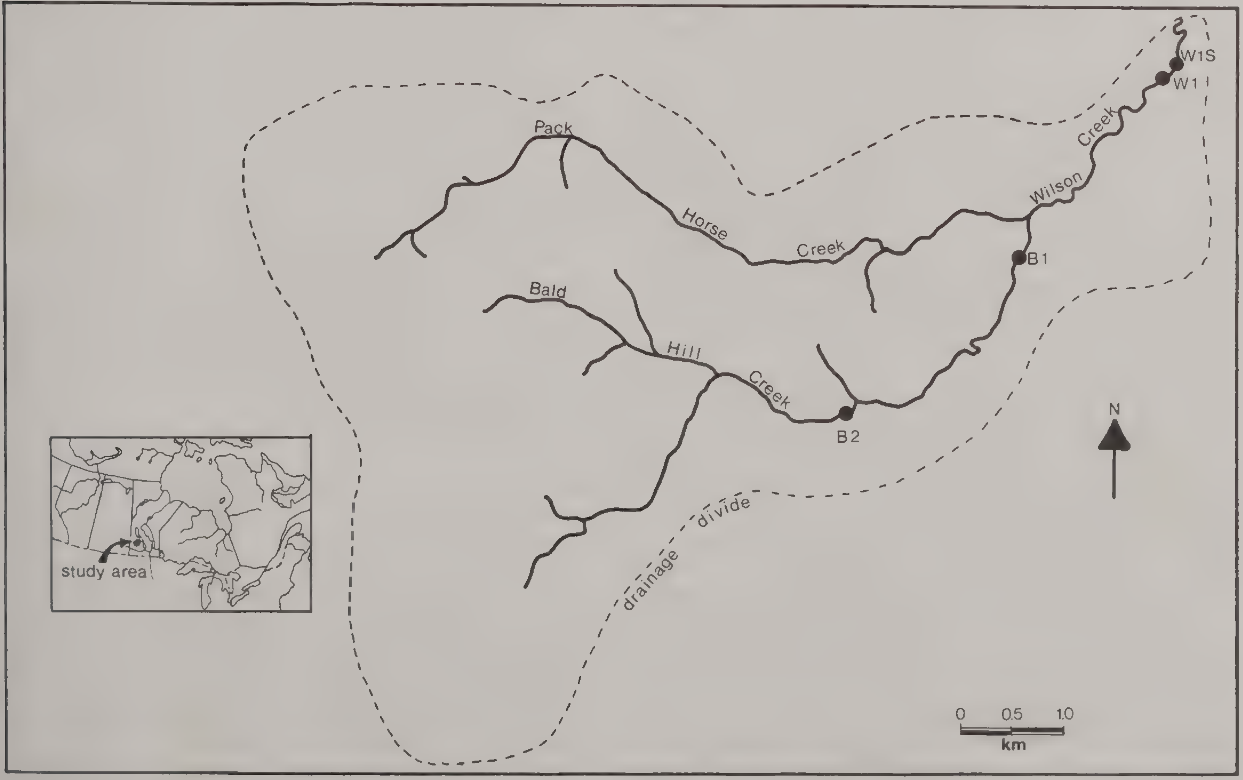

FIG. 1. Wilson Creek, Manitoba, showing locations of sampling sites (B1, B2, W1, and W1S).

TABLE 1. Hydrologic characteristics of sampling stations on Wilson Creek and summary data for selected orders of aquatic insects (1986 and 1987).

	W1	W1S[a]	B1	B2
Drainage area above station (km^2)	22.1	22.1	11.1	8.6
Slope of reach (%)	1.0	2.5	2.7	3.6
Median bed material size (cm)	4.0	10.0	22.0	34.0
Bankfull conditions				
Width (m)	4.3	4.3	3.5	3.8
Depth (m)	0.35	0.30	0.30	0.31
Discharge (m^3/s)	2.7	2.7	1.6	1.6
Tractive force (kg/m^2)	3.5	7.5	8.4	10.8
Bed paving material at incipient motion (%)[b] during				
bankfull discharge	80	40	50	20
moderate spates	10	0	0	0
low flow	0	0	0	0
Colwell's index of predictability using				
flow regime	0.42	0.42	0.42	0.42
substrate movement	0.38	0.70	0.66	0.72
Number of taxa of Ephemeroptera, Plecoptera, and Trichoptera	25	29	25	24
Mean density (no./m^2) (May to October) of Ephemeroptera, Plecoptera, and Trichoptera				
1986	1615	2209	3535	5935
1987	4062	4636	4405	5594

[a] As constructed.

[b] When adjusted for stability of shale particles.

at incipient motion. Tractive force (τ) is calculated as τ (kg/m^2) = depth (m) · gradient of reach (%) · 1000 (specific weight of water).

To calculate critical tractive force for a given discharge, and thus the size of streambed substrate at incipient motion, stage/discharge relationships were required. Discharge was monitored continuously near station W1 (Station No. 05LJ033, Water Survey of Canada) and periodically at the other reaches over several stage heights (depths of flow); peak discharge was estimated from the relationship between drainage area and bankfull discharge (discharge that fills the stream channel) (Newbury 1984). Mean depth of flow was measured at 0.5-m intervals across the stream along several transects. Average gradient of each reach was measured using a surveyor's level and elevation rod. Average slope (percent) was calculated as the change in elevation over approximately 60 m of stream reach.

Streambed paving materials were characterized for each reach by selecting 96 examples of bed paving material while walking along the thalweg. Length, width, and height of each stone was measured, and mean particle size (θ_{50}) was calculated (θ_{50} = L (cm) + W (cm) + H (cm)/3), ranked, and arranged in a cumulative frequency curve for each station (Fig. 2). From the frequency curve, the percentage of bed paving substrate at incipient motion could be estimated for any given discharge.

Several methods were used to characterize the Wilson Creek flow regime. First, direct examination of the hydrograph yielded the average annual frequency and seasonality of discharge. Second, the number of days or years in which a given flow was equalled or exceeded was calculated using a cumulative percent frequency graph. Third, the predictability, contingency, and constancy of flows were calculated using Colwell's index of predictability (Colwell 1974). Predictability is the variation among successive periods in the pattern of periodic phenomena. When variation is low, predictability is high. Predictability is dependent upon constance and contingency. Constancy is a measure of how uniform an environment is. A regulated stream often has high constancy of flow and therefore is highly predictable. Contingency is a measure of how conditions vary. If flows are high at exactly the same time each year, the contingency is high and therefore the predictability is high. Colwell's index of predictability for each station of Wilson Creek was calculated using two parameters obtained from the historical flow record. The first parameter was discharge, the method used by Resh et al. (1988). The second parameter used to calculate the index was the percentage of days in which substrate movement was accompanied by reduced insect densities.

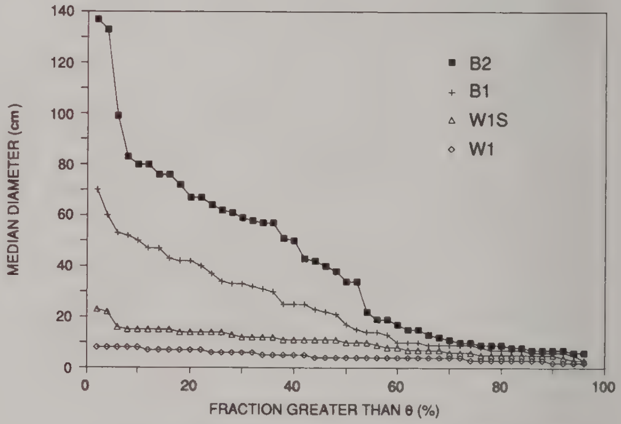

FIG. 2. Cumulative percent frequency of sample reach bed paving substrate in Wilson Creek.

Invertebrate Sampling

Immature aquatic insects were collected using a modified Hess sampler with a 400-μm-mesh and a sampling area of 0.10 m^2 (Waters and Knapp 1961). Large substrates were cleaned and removed and the remaining substrate sampled to a depth of at least 5 cm. Five benthic samples were collected from riffles in each reach monthly from May to October and following spates in 1986 and 1987. Samples were also collected before (March) and after the spring peak discharge period in 1987 and 1988. All samples were preserved in 4% formalin and later transferred to 70% ethanol. Mayflies, stoneflies, and caddisflies were identified to genus and species where possible; remaining specimens were identified to order.

To assist in life history interpretation and species identification, adult insects were collected using 1-m^3 emergence traps (Flannagan 1978). Two traps were set at each of stations W1 and B1 in 1986 and were sampled on Tuesday and Friday from mid-May through late September. Samples were collected using an aspirator and were preserved in 70% ethanol.

Statistical Analyses

Relationships between discharge, substrate movement, and benthic insect densities were examined for each station and for all stations combined (with station included and with station excluded in the analysis), using polynomial regression analysis. Analysis of covariance (ANCOVA) was used to compare regressions between stations using SAS (SAS Institute Inc. 1985). Changes in density of benthic insects at each station 1 d before and 2 d after spates in June and September 1986 were tested using analysis of variance (ANOVA), with means compared using the least squared means option in SAS (SAS Institute Inc. 1985).

Sample variances were usually larger than the means, and the two were correlated ($r = 0.9912$, 9 df, $p < 0.001$), so the data were log transformed ($\log_{10}$ number/m^2 + 1) (Elliot 1973). This transformation removed the correlation between the means and variances ($r = 0.365$, 9 df, $p > 0.05$). Discharge data were normalized using the $\log_{10}$ transformation, while the angular transformation ($\sin^{-1}\sqrt{\% / 100}$) was used to normalize the percentage of bed paving material at incipient motion.

Simpson's index, as modified by Pielou (1969), was used to compare spatial and temporal trends of community diversity. Czekanowski's coefficient of similarity (Hellawell 1978) was used to compare spatial and temporal similarity of species and their relative contribution to the community. A high value of Czekanowski's similarity index indicated similar communities. In each analysis, data from before (March) and after (April) peak discharge in 1987 and before and after June and September 1986 spates were used.

Results

Hydrological and Physical Parameters

Station B2 had the largest median streambed material size, followed by stations B1, W1S, and W1 (Table 1) (Fig. 2). Tractive force at bankfull discharge was similarly ranked. Discharge was highly correlated with percentage of bed paving substrate at incipient motion at each station ($p < 0.001$). The shale substrate at station W1 was more easily set in motion with increased discharge when compared with the other stations (ANCOVA; $p = 0.0006$) whereas no difference in this relationship occurred among the other stations ($p > 0.5$).

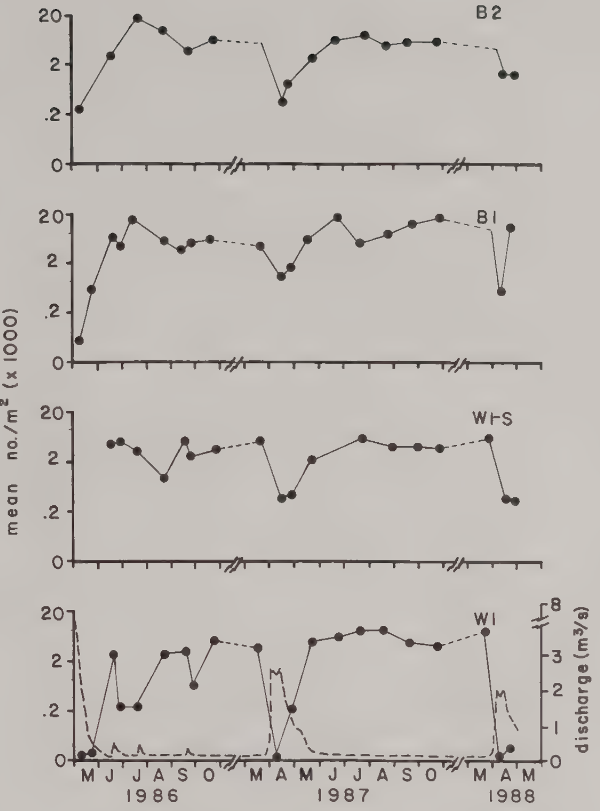

FIG. 3. Seasonal trends of discharge at station W1 (Environment Canada 1965–89) and aquatic insect abundance at stations B2, B1, W1S, and W1 on Wilson Creek, 1986–88.

The 1986 spring bankfull discharge (7.7 m^3/s) (Fig. 3) moved the largest proportion of streambed paving material at station W1 (80%) and the least at station B2 (20%; Table 1). Historically, in Wilson Creek this discharge was equalled or exceeded in 67% of the years, but it only occurred in 0.8% of the days, and only once per season on average. Summer and fall spates of 1986 resulted in 12 and 8%, respectively, of streambed paving material at incipient motion at station W1, but no movement of substrate at the other stations (Table 1). Historically, June spates occurred less frequently and had less duration than September spates.

Benthic Insects

Total densities declined during spring at all stations; higher densities occurred throughout the rest of the season (Fig. 3). Reductions in benthic densities also occurred at station W1 during June, July, and September 1986; however, a similar pattern was not observed in 1987. Periods of low discharge and subsequent high substrate stability were accompanied by high total insect density whereas increasing intensity of discharge and subsequent movement of substrates were accompanied by lower insect densities (Fig. 4).

To further examine this trend, total insect density for the whole study period was regressed against discharge and substrate movement data (Fig. 5). Insect densities remained relatively constant with increasing discharge until approximately 0.4 m^3/s, after which densities declined (Fig. 5A). Substrate movement of approximately 10% was accompanied by reduc-

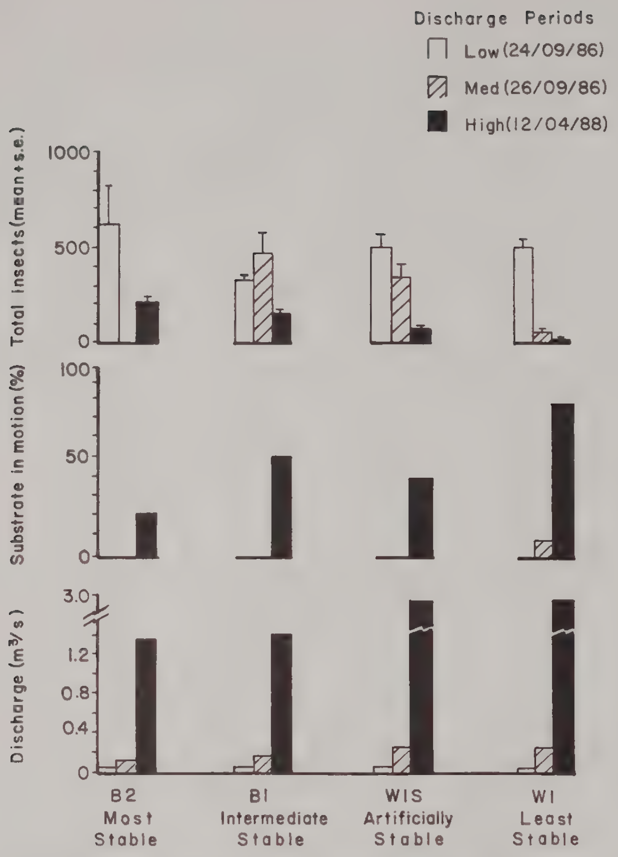

FIG. 4. Aquatic insect abundance, substrate in motion, and discharge at Wilson Creek stations B2, B1, W1S, and W1 during representative low, medium, and high discharge periods.

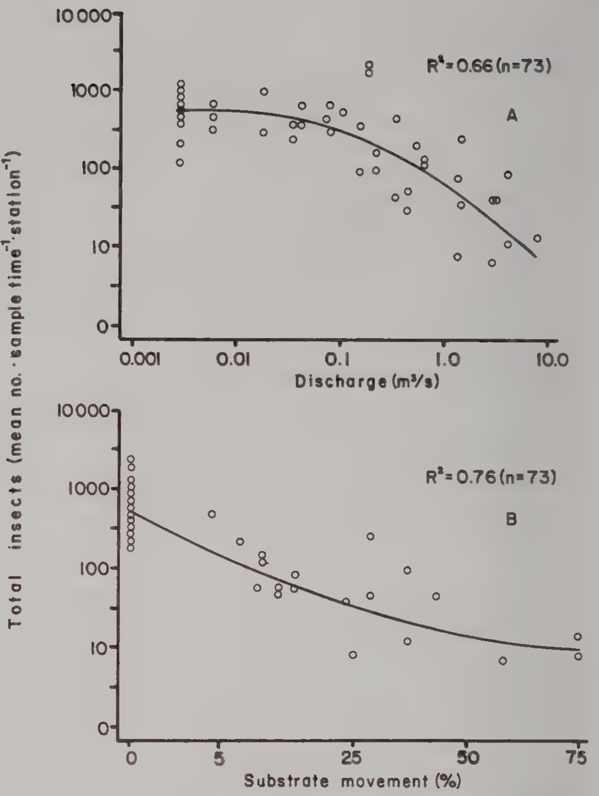

FIG. 5. Second-order polynomial regressions between insect abundance (mean total number of insects in each station) and (A) discharge and (B) percentage of streambed paving material at incipient motion using combined data for all sample periods at Wilson Creek stations B2, B1, W1S, and W1.

tion in density (Fig. 5B). Aquatic insect densities tended to decline with increasing discharge and percentage of substrate in motion in all groups except for Chironomidae at stations B1 and B2 and "others" (black flies, beetles) at station B2 (Table 2). Substrate stability accounted for more variation than discharge, except for Plecoptera (both models) and Trichoptera (station effect included).

In June and September 1986, samples were taken 1 d prior to and 2 d after moderate spates. The summer and fall 1986 spates had relatively little effect on insects in the stable artificial riffle (W1S) compared with the dramatic reductions of insects at the unstable natural reach (W1), even though both stations were subjected to equal discharge (Table 3). All insect groups at station W1 (except "others") showed highly significant ($p < 0.001$) reductions in densities; Ephemeroptera experienced the largest reduction (94%). At the other stations, only Ephemeroptera at station B1 were statistically significant ($p < 0.05$). In this study, a 40–50% change in density was required before it was statistically significant ($p < 0.05$).

Species diversity at stations W1S and B1 was relatively constant over the periods examined (Fig. 6). At station W1, diversity was reduced following the April and September spates, but not following the June spate. Czekanowski's coefficient of similarity indicated that station W1 was least similar to other stations following the spring, summer, and fall spates.

Wilson Creek had a Colwell's predictability index (P) of 0.42, a constancy index (C) of 0.13, and a contingency index (M) of 0.29 when discharge was used to characterize the flow regime (1965–85) (Table 1). A predictability of 0.31 is considered highly unpredictable (Resh et al. 1988); therefore, Wilson Creek has a relatively unpredictable flow regime.

Colwell's index of predictability was calculated using the percentage of days (1965–85) in each month that produced flows great enough to move at least 10% of the bed paving material (average of the June and September 1986 spates). Station W1 was highly unpredictable ($P = 0.38$, $C = 0.12$, $M = 0.26$), B1 was moderately predictable ($P = 0.66$, $C = 0.12$, $M = 0.54$), and stations W1S ($P = 0.70$, $C = 0.09$, $M = 0.61$) and B2 ($P = 0.72$, $C = 0.10$, $M = 0.62$) were highly predictable. Thus the largest difference between stations was in the contingency index. The more stable stations had substrate movement at the same time each year whereas the more unstable stations had more variation in the timing of substrate movement.

Discussion

Reductions in insect density of 90% may seem surprising because only 8% (September) and 12% (June) of the bed paving materials were at incipient motion. However, only streambed paving material was measured in this study; smaller particles that make up the streambed and are set in motion during spates were not measured. The addition of these smaller particles to the analysis would have revealed a larger measure of total substrate in motion. The use of bed paving material at incipient motion as a measure of stream reach substrate stability is pre-

TABLE 2. Second-order negative polynomial regression coefficients (r^2) for hydrological and aquatic insect density relationships from sample stations on Wilson Creek, 1986–88. For combined data, first value ignores station effect and second value (in parentheses) includes station effect in model. Relationships significant ($p < 0.05$) unless denoted by ns.

Model	W1	W1S	B1	B2	All stations combined
1. BPS vs. discharge[a]	0.97	0.95	0.94	0.93	—
2. Ephemeroptera vs. discharge	0.74	0.69	0.54	0.51	0.57 (0.67)
Ephemeroptera vs. BPS	0.85	0.73	0.68	0.84	0.77 (0.81)
3. Plecoptera vs. discharge	0.88	0.75	0.71	0.79	0.74 (0.82)
Plecoptera vs. BPS	0.84	0.58	0.59	0.69	0.67 (0.75)
4. Trichoptera vs. discharge	0.85	0.56	0.61	0.48	0.47 (0.81)
Trichoptera vs. BPS	0.77	0.60	0.52	0.51	0.55 (0.78)
5. Chironomidae vs. discharge	0.66	0.35	0.18 ns	0.0039 ns	0.28 (0.45)
Chironomidae vs. BPS	0.76	0.51	0.26 ns	0.21 ns	0.33 (0.55)
6. Others[b] vs. discharge	0.63	0.37	0.32	0.23 ns	0.33 (0.52)
Others vs. BPS	0.68	0.43	0.31	0.25 ns	0.44 (0.55)
7. Total insects vs. discharge	0.83	0.70	0.78	0.66	0.66 (0.81)
Total insects vs. BPS	0.89	0.77	0.73	0.65	0.76 (0.84)

[a] BPS = % bed paving substrate at incipient motion; discharge = m^3/s.

[b] Others = black flies and beetles.

TABLE 3. Effects of 23 June and 25 September 1986 spates on aquatic insect densities (no./m^2 ± SE) in Wilson Creek. Aquatic insect densities transformed using $\log_{10}$ (no./m^2 + 1) for ANOVA. When ANOVA were significant, means were compared using least squared means (LSMeans in PROC GLM, SAS Institute Inc. 1985). ns = $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Group	Stn	Density 23 June (pre)	Density 25 June (post)	% change	Density 24 Sept. (pre)	Density 26 Sept. (post)	% change
Ephemeroptera	W1	816.0 (155.6)	46.0 (10.8)	−94.4***	800.0 (195)	74.0 (12.5)	−90.8***
	W1S	1244.0 (87.3)	998.0 (159.1)	−19.8 ns	390.0 (88.7)	420.0 (151.3)	+7.7 ns
	B1	1054.0 (183.3)	596.0 (78.0)	−43.4*	532.0 (71.7)	260.0 (54.4)	−51.1*
Plecoptera	W1	378.0 (79.8)	76.0 (16.9)	−80.0***	2390.0 (331.3)	606.0 (92.0)	−74.6***
	W1S	548.0 (108.8)	502.0 (128.2)	−8.4 ns	2690.0 (435.5)	1516.7 (1006)	−43.6 ns
	B1	528.0 (135.3)	310.0 (61.0)	−41.2 ns	1174.0 (202.8)	1700.0 (28.3)	+44.8 ns
Trichoptera	W1	51.9 (9.16)	3.99 (2.45)	−92.5***	342.0 (61.5)	70.0 (12.5)	−79.5***
	W1S	243.9 (33.5)	209.8 (52.7)	−14.0 ns	384.0 (103)	786.7 (236.8)	+105*
	B1	105.9 (26.6)	86.0 (14.3)	−18.8 ns	1290.0 (154.3)	2622.0 (800.1)	+103 ns
Chironomidae	W1	932.0 (228.4)	236.0 (74.1)	−74.7***	1030.0 (290.6)	110.0 (28.8)	−89.3***
	W1S	852.0 (233.8)	1092.0 (220)	+28.2 ns	1060.0 (331.4)	520.0 (120.1)	−49.0 ns
	B1	3050 (326.2)	1542 (227.3)	−49.0 ns	34.0 (6.78)	192.0 (37.6)	+464***
Others[a]	W1	236 (51.8)	186 (55)	−21.2 ns	462.0 (110.2)	82.0 (22.7)	−82.5***
	W1S	862 (176)	1428 (629)	+65.7 ns	568.0 (170)	266.7 (46.7)	−53.0 ns
	B1	5402 (1472)	3252 (1551)	−39.8 ns	154.0 (15.0)	172.0 (28.5)	+11.7 ns
Total	W1	2434 (248)	554 (66.1)	−77.2***	5020.0 (698.7)	942.0 (81.1)	−81.2***
	W1S	3860 (357)	4362 (630)	+13.0 ns	5092.0 (895.0)	3510.0 (1120)	−31.0 ns
	B1	10178 (1676)	5838 (1848)	−42.0 ns	3184.0 (318.4)	4946.0 (1039)	+55.0 ns

[a] Others = black flies and beetles.

ferred because these materials are a result of channel forming processes which act on a relatively long geologic time scale (Newbury 1984) and thus are important determinants of aquatic insect habitat. This method is also preferred because the parameter is relatively easily measured with a minimum of equipment and because the method is suitable for use in remote regions.

Indicator Species of Substrate Stability

The trichopteran *Glossosoma intermedium* was numerically dominant at stations B1 and B2 whereas it was rare at the other two stations (Cobb et al. 1991b). This species may have been resistant to spring spates at stations B1 and B2 because in late fall, it aggregates on the lower rear face of large cobbles and boulders (D. G. Cobb, unpubl. obs.). The selective advantage of this activity is unknown, but it may allow individuals to avoid the hydraulic stress associated with spates. Because over 80% of the streambed paving materials in the unstable reach (W1) were moved during spring spates, this behavioral adaptation is of little advantage in this habitat. However, surprisingly few individuals of *G. intermedium* colonized the artificial riffle, perhaps because the species is not mobile (e.g. Anderson 1967). A larval transplant experiment would determine whether

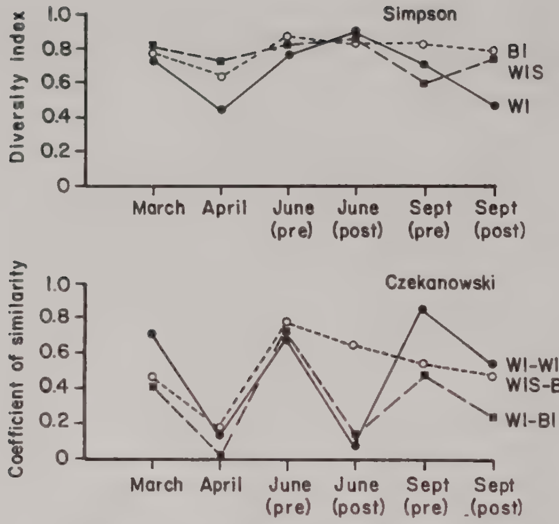

FIG. 6. Seasonal trends in Simpson's index of diversity and Czekanowski's index of similarity before and after spates at each Wilson Creek station, 1986–87.

G. intermedium could survive in this newly created habitat. *Glossosoma intermedium* is distributed in the lower reaches of other Manitoba escarpment streams, but its greatest abundance was recorded on large stable substrates (Cobb et al. 1984). Aggregation and low dispersal are two characteristics that make *G. intermedium* and other species with similar characteristics good indicators of substrate stability.

Disturbance and Stream Insect Communities

In the absence of disturbance, stream insect community structure may be controlled by biotic factors (e.g. Peckarsky 1979). Such communities are characterized by low species turnover, stable species richness, and "high resistance" to perturbation. Alternatively, communities controlled by stochastic factors (e.g. Gray and Fisher 1981; Reice 1985; Sagar 1986; Doeg et al. 1989) have high species turnover (i.e. "low resistance" to perturbation) and rapid recolonization to predisturbance levels (i.e. "high resilience", Lake and Doeg 1985). In studies where substrates were experimentally or naturally disturbed, both abundance and species richness declined, the former to a greater extent than the latter (e.g. Reice 1985). Each study stream recovered to predisturbance levels within a few to several tens of days, presumably by recolonization through drift, oviposition by adults, or life history adaptations (Scrimgeour et al. 1988). Wilson Creek was characterized by low resistance when greater than 10% of bed paving material was in motion, but the insect community was resilient, as abundance and diversity rebounded at all stations during extended periods of low flow. Life history adaptations of the majority of species at station W1 favored survival in an environment where substrates are disturbed in spring and fall, but were not so favored in the event of unpredictable summer spates (Cobb et al. 1991a). On average (1965–85) in Wilson Creek, there are annually six summer–autumn spates of similar magnitude to that of September 1986. During the present study, there were only three moderate summer–autumn spates. An "average" year, with a higher frequency of spates, may result in a more depauperate fauna at station W1 than occurred in this study. When interpreting results of a study, it is important to examine flow data in relation to the historical average flow regime. If station W1 had been sampled only in 1987, when there were no summer–fall spates, seasonal abundance and species diversity would have appeared quite similar to other stations sampled (Fig. 3). This was not the case in 1986, and probably even less so during a year with an average of six spates capable of moving 10% of bed paving material at station W1.

Substrate Stability and Disturbance

What constitutes a disturbance and what characteristics are required to analyze disturbance with respect to aquatic insect habitat? Resh et al. (1988) used the definition of disturbance from Pickett and White (1985): "any relatively discrete event in time that disrupts ecosystem, community, or population stucture, and that changes resources, availability of substratum, or the physical environment." They showed that historical data can be analyzed to describe the flow regime of any drainage basin where sufficient data records exist and that the insect fauna may be characterized in light of the flow regime. This is an important step toward a better understanding of the abiotic factors affecting aquatic organisms. The results of our study suggest that the variability in effects of discharges on stream insects depends upon the stability of substrates associated with discharge. We suggest that an analysis of the predictability of flow regimes on aquatic insect communities include the effects of substrate stability. Regionally, reaches within and among unregulated streams have similar predictability of discharge because they are situated within the same climate, which normally dictates the timing of discharge above base flow. For example, in north-continental climates, there are often large annual spring peak flows caused by snowmelt; these are followed by several smaller spates related to summer rain storms. Autumnal flow increases are related to rain storms coupled with a reduction in evapotranspiration. However, within the same geographic region, based upon geological features, substrate movement within reaches of streams and among streams can be very different for a given discharge; this would result in differing predictability indices (Table 1).

Implications of Discharge/Substrate Stability Relationships

An important determinant in ecological studies (Resh et al. 1988), disturbance could have potential implications in the interpretation of impacts of anthropogenic activities on aquatic ecosystems. Pre- and postdevelopment monitoring requires an understanding of natural variability in populations of aquatic organisms within potentially impacted areas and in control sites. If discharge related substrate movement resulted in more (or less) frequent disturbance of the treatment station than the control site, this would likely result in differences in abundance and/or species diversity between sites. Conclusions of detrimental effects (or no effect) could be reached when the opposite may be true. Controlling for the effects of discharge/substrate stability relationships on aquatic biota at the two sites through predevelopment baseline modelling of insect/substrate stability relationships, or the construction of hydrologically similar "index" riffles, would lead to a better understanding of the real impact of the anthropogenic activity.

Conclusion

Recognition of the importance of historical flow data (Resh et al. 1988; McElravy et al. 1989; Rader and Ward 1989) is an important development in the field of aquatic ecology. Frequency, intensity, and predictability of disturbance may (1) affect productivity, nutrient cycling, and spiralling, (2) determine life history and behavioral strategies, and (3) influence species composition (see review by Resh et al. 1988). With the recent emphasis on environmental impact assessment and mitigation and fisheries enhancement and rehabilitation, an understanding of natural processes which determine aquatic habitats is required. It is hoped that the results of this study contribute towards that understanding. By expanding the scope of the present study, it will be possible to determine whether the aquatic insect/hydrological relationships derived for Wilson Creek are applicable to other rivers and streams.

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Optimal Harvesting Policy for Chub Mackerel (*Scomber japonicus*) in Japan under a Fluctuating Environment

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Matsuda, H., T. Kishida, and T. Kidachi. 1992. Optimal harvesting policy for chub mackerel (*Scomber japonicus*) in Japan under a fluctuating environment. Can. J. Fish. Aquat. Sci. 49: 1796–1800.

Temporal fluctuation in the reproduction rates of chub mackerel (*Scomber japonicus*) was estimated for the period 1971–86, when stock abundance of chub mackerel varied from a high to low level. To estimate the effect of various harvesting policies on stock conservation and the long-term catch, six harvesting policies were considered: (1) actual catch data during 1975–88, (2) 1/3 constant rate of exploitation, (3) low maximal catch amount, (4) 1/5 constant rate of exploitation, (5) prohibition of fishing when the stock is rare, and (6) constant-escapement policy. Using the data of reproduction rates during 1975–85, the final stock abundance at 1988 and the total catch amount during 1975–88 under each harvesting policy are calculated. We concluded that harvesting policies (4), (5), and (6) were effective for the stock conservation and that policies (2), (5), and (6) were effective for increasing the total catch.

On a estimé la fluctuation temporelle du taux de reproduction du maquereau blanc (*Scomber japonicus*) pour la période 1971–1986, pendant laquelle l'abondance du stock est passée d'un niveau élevé à un niveau bas. Pour estimer l'effet des diverses politiques de capture sur la conservation du stock et les prises à long terme, on a examiné six politiques : 1) données réelles sur les captures de 1975 à 1988, 2) taux d'exploitation constant de 1/3, 3) prises maximales fixées au niveau le plus bas, 4) taux d'exploitation constant de 1/5, 5) interdiction de la pêche en cas de rareté et 6) maintien d'un niveau constant des échappées. À partir des données sur les taux de reproduction de la période 1975–85, on a calculé l'abondance du stock en 1988 et le montant total des captures de 1975 à 1988 en fonction de chacune des politiques. Nous concluons que les politiques 4, 5 et 6 sont efficaces en ce qui concerne la conservation du stock, et que les politiques 2, 5 et 6 sont efficaces en ce qui concerne l'augmentation du total des captures.

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Chub mackerel (*Scomber japonicus*) was very abundant in Japanese waters during 1965–75 but declined thereafter while the Japanese sardine (*Sardinops melanosticta*) increased in abundance. Although there are some indications that the sardine will soon decrease (the annual catch in 1990 was less than in 1989), the chub mackerel population is still at a very low level. Because the purse-seine and dip-net fisheries appear to markedly decrease the recruitment of chub mackerel, it is important that the exact rate of exploitation be estimated.

It is well known that the reproduction rates of pelagic fishes fluctuate from year to year. Recruitment may be much larger than the annual catch in number under favorable environmental conditions, and stock abundance may decrease even while fishing is prohibited if the environment is unfavorable. Although it would seem intuitively difficult to find an optimal fishing policy under a fluctuating environment, the constant-escapement policy (Reed 1979) is theoretically sound. Ruppert et al. (1985) showed that under such a policy, assuming stochastic egg recruitment, average catches would be higher than under a constant catch-effort policy. They used an age-structured population and estimated an average catch amount with Monte-Carlo simulations. In Japan, the constant-escapement policy was not practiced in spite of a very low abundance of chub mackerel.

The main aim of this paper is to show differences in the stock abundance and the total catch amount under various harvesting policies. We estimated annual reproduction rates of the chub mackerel stock from 1971 to 1985, using estimated yearly egg production of chub mackerel from 1971 to 1988, and annual catch data for the Pacific coast of eastern Japan. We also calculated the final stock abundance in 1988 and the total catch amount during 1975–88 under each harvesting policy.

Methods

Data

From data on average body weight of an adult fish (W, grams) and the average number of spawned eggs in a year per adult female (K), we can estimate approximately the stock abundance in biomass of the adult mackerel (N_t, millions of tons) as

$$(1)\quad N_t = 2E_tW/K \times 10^{-6} \quad \text{for } t = 1971, 1972, \ldots, 1988$$

where E_t is the estimated yearly egg production of chub mackerel in year t, and we assume that the sex ratio is 0.5. We assume that $W = 760$ g and $K = 200\,000$. The estimated yearly egg production, E_t, is shown in Table 1.

If we used data on egg production in a day, the average body weight at age a, the average number of annual spawned eggs per female at age a, the ratio of spawning female at age a in a day, and the assumption that relative catchability is independent of age (no gear preference in fish age), then we could more exactly estimate the adult stock abundance in biomass by adopting the "egg production method" (see Lasker 1985). Unfortunately, there are no Japanese data on clutch size or the frequency of spawning bouts. Thus it is assumed that stock abundance is roughly proportional to egg production.

An age-structured model was sometimes less appropriate for catch-effort data than a simple model (Ludwig and Walters 1985). Thus, in this paper, we simply ignored the age dependence of adult mackerel for such characters as natural mortality, batch fecundity, and body weight for calculation of the stock abundance in number of all adults.

Estimation of Reproduction Rate

We assumed that the survival rate of adult mackerel (S) is constant independent of year t and age of fish, that fish mature at 3 yr old, and that catch of immature fish is zero, although the purse-seine fishery in fact exploits immature fish. The stock abundance at year t satisfies the following equation:

$$(2)\quad N_t = SN_{t-1} + R_{t-3}N_{t-3} - C_t \qquad \text{for } t = 1974, 1976, \ldots, 1988$$

where C_t is the annual catch amount in biomass as shown in Table 1. The quantity R_t, the "reproduction index," is the number of adult fish surviving which were spawned by one adult fish from year t to 3 yr later. In Japan, both adult and young mackerel are caught by purse seine before the spawning period (from March to May) off the northeastern coast, and adults are caught by dip net during the spawning period. Because the catch by dip net is much smaller than that by purse seine, we ignore the catch during the spawning period in eq. (2). Note that the stock abundance before purse-seine fishing is $N_t + C_t$, and that the rate of exploitation at year t is $C_t/(N_t + C_t)$.

In this model, an adult mackerel survives until the next year with probability S, and then the average reproduction rate is $<R>/(1 - S)$ where $<R>$ is the geometric average of R_t. If $C_t = C$ for every year (constant) and $R_t = 1 - S + C/N_t$ for every year, then the stock abundance maintains an equilibrium level.

Hightower and Grossman (1985) considered an age-structured model incorporating fluctuating reproduction for the anchovy (*Engraulis capensis*), Atlantic menhaden (*Brevoortia tyrannus*), and Pacific ocean perch (*Sebastes alutus*) in which they assumed that the survival rate from egg to 1-yr-old fish is

$$(3)\quad R_t = Z_t f(E_t)$$

where Z_t is a random variable and $f(E_t)$ is a deterministic density-dependent function of egg production at year t. They also assumed the survival rate after 1 year old is constant and a constant exploiting effort from year to year. In the sardine, both the maturation age and the survival rate of adult fish fluctuate from year to year and both depend on the stock abundance level (Wada 1988).

We further assumed that the reproduction index depends on both the environment and the stock abundance, according to Ricker's (1954) model:

$$(4)\quad R_t = \exp[b_t - kN_t] \qquad \text{for } t = 1971, 1972, \ldots, 1985$$

where b_t is the time-dependent Malthusian parameter and k is the intensity of density effect. The carrying capacity corresponds to b^*/k where b^* is the geometric average of b_t. Using eq. (1), we can express

$$(5)\quad \begin{aligned} Z_t &= \exp[b_t - b^*] \\ f(E_t) &= \exp[b^* - 2kE_tW/K \times 10^{-6}]. \end{aligned}$$

Thus, eq. (4) satisfies eq. (3), which was analyzed by Reed (1979) and Hightower and Grossman (1985). If values of the parameters S and k are given, then we obtain the year-dependent reproduction index R_t and b_t as

$$(6)\quad \begin{aligned} R_t &= (N_{t+3} - SN_{t+2} + C_{t+3})/N_t \\ b_t &= kN_t + \log[(N_{t+3} - SN_{t+2} + C_{t+3})/N_t] \end{aligned} \qquad \text{for } t = 1971, 1972, \ldots, 1985.$$

Simulation of Total Catch and Final Stock

Using values of S, k, b_t ($t = 1971, 1972, \ldots, 1985$), and the initial stock abundance $N_{1971} = 1363$ (10^3 tons), we calculated the total catch amount during 1975–88 $\Sigma_t C_t$, and the final stock abundance N_T ($T = 1988$) under various harvesting policies. A harvesting policy is described as

$$(7)\quad C_t = C(N_c; h(N_t), C_m) \qquad \text{for } t = 1975, 1976, \ldots, 1988$$

where $h(N_t)$ is a positive function of N_t, and

$$(8)\quad C(N_c; h(N_t), C_m) = \begin{cases} 0 & \text{if } N_t < N_c \\ \min(h(N_t), C_m) & \text{if } N_t \geq N_c. \end{cases}$$

Here, we call C_m and N_c "maximal catch amount" and "critical stock level," respectively. We consider the following six policies:

Policy 1: C_t = actual catch amounts as shown in Table 1
(9a) Policy 2: $C_t = C(0; N_t/3, 2000)$
(9b) Policy 3: $C_t = C(0; N_t/3, 1000)$
(9c) Policy 4: $C_t = C(0; N_t/5, 2000)$
(9d) Policy 5: $C_t = C(1500; 2N_t/5, 2000)$
(9e) Policy 6: $C_t = C(1500; N_t - 1500, +\infty)$
for $t = 1975, 1976, \ldots, 1988$.

We call these policies (1) actual, (2) 1/3 constant rate of exploitation, (3) low maximal catch, (4) 1/5 constant rate of exploitation, (5) positive critical stock level, and (6) constant escapement. Policy (6) satisfies the condition of the optimal harvesting policy proposed by Reed (1979).

Results

From 1971 to 1985 under the assumption of $S = 0.5$ and $k = 0.0006$, the reproduction index b_t fluctuated enormously from year to year (Table 1).

Under policies (1), (2), and (3), the final stock level is very low (Fig. 1). Policy (1) gives the worst result of these policies because the resultant rate of exploitation at low stock levels after 1983 was much larger than 1/3, which is similar to the constant catch amount policy rather than the constant rate of exploitation policy. The effect of stock conservation at a high stock level of policy (3) on increase in the final stock level is very small. Under policies (4), (5), and (6), the final stock abundance was maintained to a higher level.

Under policies (1), (3), and (4), the total catch amounts were less than 9 million tons whereas under policies (2), (5), and (6), the total catches more than 10 million tons (Fig. 2). Note that under policies (5) and (6), there were long intervals of prohibition. Catch was prohibited from 1981 to 1985 under policy (5) and from 1981 to 1986 under policy (6).

Also note that under policy (4), there was a larger annual catch in 1988 than the actual catch because stock abundance was much higher than the actual level due to stock conservation.

TABLE 1. Yearly change in catch amount, egg production, stock abundance, reproduction index, Malthusian parameter, and rate of exploitation.

Year (t)	Catch amount (C_t, 10^3 tons)[a]	Egg production (E_t, 10^{12})[b]	Stock abundance (N_t, 10^3 tons)	Reproduction (R_t)	Malthusian parameter (b_t)	Rate of exploitation ($C_t/(N_t + C_t)$)
1971	1001	179.4	1363	4.917	1.675	0.423
1972	901	432.0	3283	1.338	0.488	0.215
1973	780	509.3	3871	1.115	0.341	0.168
1974	861	1023.5	7779	0.176	−1.273	0.100
1975	929	967.4	7352	0.531	−0.192	0.112
1976	679	962.2	7313	0.182	−1.266	0.085
1977	860	547.6	4162	0.611	−0.243	0.171
1978	1300	616.4	4685	0.228	−1.199	0.217
1979	1100	338.4	2572	0.250	−1.233	0.300
1980	860	390.6	2968	0.018	−3.856	0.225
1981	480	272.4	2070	0.281	−1.145	0.188
1982	260	186.5	1418	0.331	−1.022	0.155
1983	370	51.5	391	2.555	0.961	0.486
1984	470	40.4	307	1.882	0.651	0.605
1985	390	30.6	232	1.054	0.067	0.627
1986	600	67.9	516	0.722	−0.295	0.538
1987	350	64.0	486			
1988	260	30.0	228			

[a] According to the catch statistics compiled by the Japan Ministry of Agriculture, Forest and Fisheries.
[b] According to the egg production surveys compiled by the Fisheries Agency of Japan.

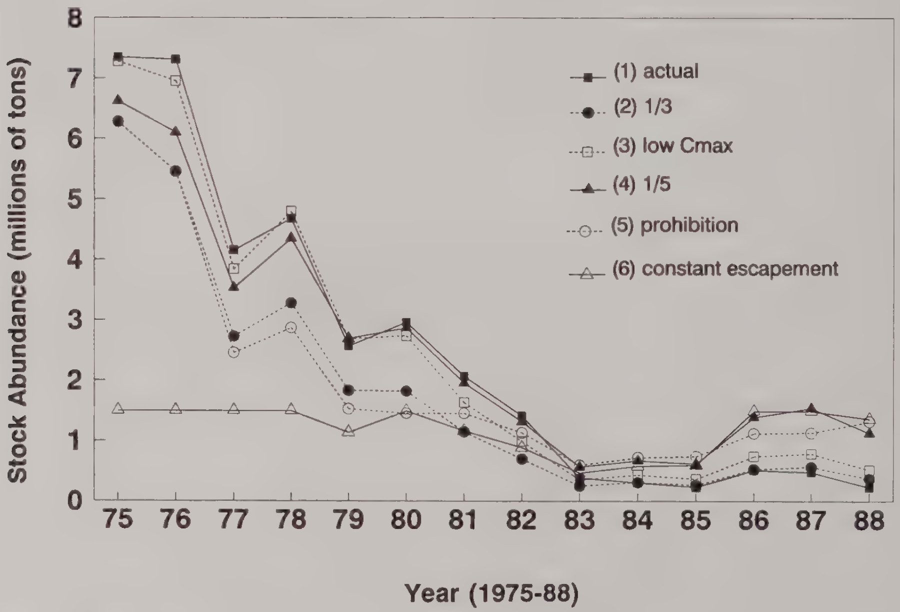

FIG. 1. Yearly change in simulated stock abundances under various harvesting policies ((1)–(6)) from 1975 to 1988.

Discussion

In Japan, the Fisheries Agency has been using the 1/3 constant rate of exploitation policy for pelagic fishes. However, this policy is not always effective for stock management. In this paper, we have shown that both the constant-escapement policy and prohibition of fishing when the stock level is less than a critical stock level are effective both for stock conservation and for increasing the long-term average catch amount for chub mackerel.

The method for estimating the reproduction index shown in this paper is simple and convenient because we use only long-term changes in egg production and catch abundance. However, estimation errors in the egg production, E_t, and/or the survival rate of adult fish, S, may lead to errors in the reproduction index. We could not obtain values of the survival rate, S, and the intensity of the density effect, k, from actual data, E_t and C_t. Only in recent years has sufficient detail (e.g. age structure, size distribution, catch of immature fish) become available in Japan for estimation of yearly change in the reproduction index.

According to our study, when the stock level was high, the rate of exploitation was less than 1/10 and the effect of catch on stock fluctuation was not so large; furthermore, density-

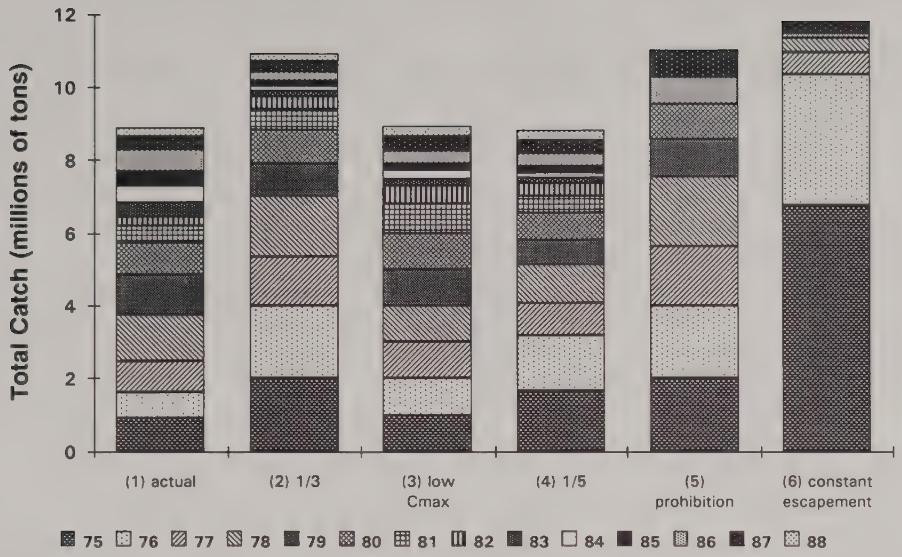

FIG. 2. Yearly change in simulated catch amounts under various harvesting policies ((1)–(6)) from 1975 to 1988. Components of annual catch amount are discriminated by hatching patterns.

dependent survivorship reduces this effect. On the other hand, at a low stock level, when density-dependent survivorship seems negligible, the rate of exploitation may become so large that the catch markedly damages the stock abundance.

Although prohibition of fishing while stock levels are low is effective in increasing the long-term catch, it may be difficult to adopt this policy because the stock abundance could decrease even while fishing is prohibited, as shown in Fig. 1, curve for policy (5), during 1979–81. The large fluctuation of stock abundance in pelagic fish populations may result in increased stock abundance even under a strong rate of exploitation or decreased abundance even under a zero-catch (prohibition) policy. Some may therefore suspect that there is no relationship between the time change in the stock abundance and the catch amount. However, our comparison of stock abundances under various harvesting policies under the same environmental conditions showed the effect of restricting fishing intensity, especially when stock abundance was low.

Table 1 shows that in recent years, the reproduction index may not have been very low. This suggests that the recent crisis of chub mackerel in Japan is not only the result of unfavorable environment but also of overexploitation.

As shown by Reed (1979), the constant-escapement policy is the best for maximizing total catch. However, this policy may not be politically feasible due to a long prohibition period. If we must avoid a large variance in annual catch due to prohibition of fishing, and if we want to assure a stable income to fishermen, then the constant-escapement policy is not suitable.

Such constraints may be incorporated into management policies if we adopt a policy maximizing geometric mean of annual catch instead of total catch (or algebraic mean of annual catch). Geometric means of catch under policies (1), (2), (3), and (4) are, respectively, 561, 498, 523, and 471 $\times$ 10^3 tons, while policies (5) and (6) have 0 geometric mean due to zero catch during prohibition. However, we should not compare geometric means under various policies if the final stocks are very different. The actual fishing policy has the highest geometric mean, but it results in an extremely low final stock.

The most suitable policy depends on the economy and market system. Recently, we have been able to save frozen fish for more than 1 yr, and Japanese have imported chub mackerel from Norway. Thus, the recent price of chub mackerel depends less on annual catch than it did several decades ago, and a short-term prohibition may not be critical. These factors may support the constant-escapement policy.

In summary, we should not only find the optimal harvesting policy but should also show an algorithm simulating annual catch and stock amount under various harvesting policies. This is useful for discussing which policy should be adopted. It is important for any pelagic fish with an enormously fluctuating reproduction rate that we can predict the final stock level and total catch under any other policy. In the case of chub mackerel in Japan, we believe that some restriction on fishing is necessary and that the 1/5 constant rate of exploitation policy can be adopted and is a suitable policy because total catch under this policy is not less than and final stock is much higher than the actual (catch and stock estimate, see Fig. 2 and 1, respectively).

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Statistical Models for the Analysis of Ageing Error

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We present statistical models for estimating the true age distribution of a population, based on multiple readings from individual fish. There are two steps to this process. The first involves estimating a classification matrix that defines the probability of assigning an age *a* to a fish when its true age is *b*. Since true age is unknown, we require an assumption related to ageing error bias; we assume that the true age is the most probable value for the observed age. True age proportions, or alternatively, true ages of fish in the sample are then estimated in the second step. Our methods allow us either to conduct both steps simultaneously or to estimate true age proportions from a previously estimated classification matrix. We illustrate our methods with data on walleye pollock (*Theragra chalcogramma*). We recommend that multiple independent readings be obtained for a subset of structures in future ageing studies and that ageing error be considered in subsequent analyses. Sample sizes must be increased with increasing ageing error to achieve a specified precision in estimates of true age proportions.

Nous présentons des modèles statistiques destinés à estimer la distribution de l'âge réel d'une population à partir de mesures multiples sur des poissons pris individuellement. La démarche comporte deux étapes. La première est le calcul d'une matrice de classification qui définit la probabilité d'assigner un âge *a* à un poisson tandis que son âge réel est *b*. Étant donné qu'on ne connaît pas l'âge réel, nous devons émettre une hypothèse concernant l'erreur systématique dans la détermination de l'âge; nous posons que l'âge réel est la valeur la plus probable de l'âge observé. Dans la seconde étape, on estime les proportions des âges réels, ou encore l'âge réel des poissons de l'échantillon. Nos méthodes nous permettent soit d'effectuer les deux étapes simultanément soit d'estimer les proportions des âges réels à partir d'une matrice de classification calculée antérieurement. Nous illustrons nos méthodes avec des données sur la goberge de l'Alaska (*Theragra chalcogramma*). Nous recommandons d'obtenir des mesures indépendantes multiples pour un sous-ensemble de structures dans les études futures sur la détermination de l'âge, et de considérer dans les analyses subséquentes l'erreur dans la détermination de l'âge. Il est nécessaire d'augmenter la taille des échantillons à mesure que l'erreur augmente pour arriver à une précision donnée dans l'estimation des proportions des âges réels.

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Catch–age data play a fundamental role in fish stock assessments (e.g. Megrey 1989) and in other studies of recruitment, growth, and mortality. Although many such analyses assume that fish ages are measured without error, this assumption is rarely met (Beamish and McFarlane 1983). Consequently, year-class strength can be incorrectly determined. Lai and Gunderson (1987) and Tyler et al. (1989) demonstrate how ageing error can lead to inappropriate yield projections and overfishing. Biased estimates of year-class strength can also mask important stock–recruit relationships and environmental correlates of year-class strength (Fournier and Archibald 1982; Hollowed et al. 1987; Myers and Drinkwater 1989; Bradford 1991).

In this paper, we present statistical models for estimating the true age distribution of a population, based on multiple age readings of individual fish. Although recent catch–age models can incorporate ageing error when it is known (Fournier and Archibald 1982; Methot 1989, 1990), general statistical methods have not been presented for assessing ageing error empirically. We describe several maximum likelihood estimation procedures; the one chosen for a particular situation depends on the input data and the specific question of interest.

Two main issues must be considered in any analysis of ageing error. The first deals with estimation of the classification matrix, which defines the probability of assigning an age *a* to a fish with true age *b*. The second issue involves estimation of true age proportions within a sample of fish, when individual ages may be assigned with error. Our analysis can address both issues simultaneously, based on a sample of structures for which two or more independent age readings are available. Other authors (e.g. Kimura and Lyons 1991) have advocated use of such data to assess reader bias and variability in order to maintain quality control.

The observed age *a* of a fish is a random variable that presumably bears some relationship to its true age *b*. Since true age is unknown, construction of the classification matrix forces us to make an assumption related to ageing error bias. In this paper, we assume that the observation *a* takes the value *b* with highest probability. We cannot assume that *a* is an unbiased estimate of *b* because observed ages must fall within the life span of the species. For example, due to truncation in the age distribution, mean assigned age will overestimate true age for the youngest fish and underestimate true age for the oldest fish.

The goal of most ageing error analyses is to estimate true age proportions. For example, if all fish in a sample have true age *b*, the observed ages typically take a range of values in the vicinity of *b*. In general, ageing error smooths differences in year-class strength, making strong year-classes appear weaker and weak year-classes appear stronger (Fournier and Archibald 1982; Rivard 1989; Kimura and Lyons 1991). The extent of smoothing is governed by the classification matrix. Mathematically, the expected value of the vector of observed age proportions is the product of the classification matrix and the vector of true proportions. This process leads to smoothing because each true proportion is distributed among adjacent ages. An error analysis seeks to reverse this process by allocating observed proportions back to the correct ages. As discussed by several authors (e.g. Pella and Robertson 1979), this is accomplished mathematically using the inverse classification matrix.

Estimation of true age proportions based on a known classification matrix represents one special case of analyses described in this paper. We also consider the need to estimate both the true proportions and the classification matrix simultaneously. Alternatively, estimates of true ages of individual fish within a sample may be required rather than the overall true age proportions. Such estimates could be obtained either from a known classification matrix or simultaneously with estimation of the classification matrix.

A naive method of estimating the true age proportions might be first to assign an age to each fish in a sample, based on multiple readings per fish, and then to estimate age proportions from these ages. This method is incorrect because the assigned age will sometimes deviate from the true age. Unless the age of each fish can be perfectly determined, the resulting age proportions still contain ageing error. The smoothing effect of ageing error is reduced, but not eliminated by such a process. We illustrate this feature in our analysis below.

The process of stock identification using mixture models (Pella and Robertson 1979; Fournier et al. 1984; Hoenig and Heisey 1987; Wood et al. 1987) has features in common with our analysis. In stock identification, a learning sample is used to relate a surrogate variable, such as a particular meristic, to the stock of origin. This relationship can then be used to estimate the proportions of fish from each stock in a sample for which only the surrogate variable is known. For the age data described here, the learning sample consists of multiple age readings; however, the true age of each fish is not known, unlike the true stock in a mixture learning sample. Consequently, our likelihood analysis differs in certain respects from that developed for stock mixtures.

No model can detect a systematic difference between observed and true ages. For example, if the ageing method fails to recognize the first annulus of each otolith, then the observed age could differ consistently by 1 yr from the true age. Our method cannot substitute for careful age validation. Rather, given reasonable assumptions about the classification matrix, it provides statistical corrections to age distributions estimated from multiple age readings.

Rigorous model formulation necessitates statistical detail. Models are presented in section 1, along with methods of statistical inference. Section 2 describes an application of our general methods to data on walleye pollock (*Theragra chalcogramma*). Reader effects are evident in the pollock data and a model tailored specifically to these data is illustrated in section 3. More general methods may be required for other data sets and we discuss several possible model extensions in section 4. To simplify the text as much as possible, some of the model equations are presented in tables. We use parentheses ( ) and brackets [ ] to denote equations in text and tables, respectively.

1. Statistical Model

1A. Concepts and Notation

Before we describe the statistical model, we must first discuss the raw data and develop model notation (Table 1). We assume that a sample of age structures has been collected from a fish stock and, in general, that multiple independent age readings have been obtained for each structure in the sample. Repeated readings by a single reader could serve as independent observations, provided that the experimental design minimizes the possibility of recognizing previously read structures. Alternatively, readings could be conducted by different readers following standardized methods. At the end of this section, we consider a more complex case, where multiple readings are obtained for all age structures in a subsample and the remaining structures are assigned at random to one of the readers.

TABLE 1. Model notation in the context of samples for which one (1) or several (2+) readers examine each age structure.

Symbol	Readers	Description
		Age descriptors
a	2 +	Observed age-class ($a = 1, \ldots, A$)
b	2 +	True age-class ($b = 1, \ldots, A$)
c	1	Observed age-class ($c = 1, \ldots, A$)
d	1	True age-class ($d = 1, \ldots, A$)
		Indices
i	2 +	Fish index ($i = 1, \ldots, I$)
j	2 +	Reader index ($j = 1, \ldots, J$)
k	1	Fish index ($k = 1, \ldots, K$)
m	1, 2 +	Parameter index for $\mathbf{\Phi}$ ($m = 1, \ldots, M$)
n	1, 2 +	Parameter index for $\mathbf{\Theta}$ ($n = 1, \ldots, N$)
		Age vectors and matrices
a_{ij}	2 +	Age-class of fish i according to reader j
$\mathbf{A}_i$	2 +	Vector $(a_{ij})_{j=1,\ldots,J}$ of observed age-classes for fish i
$\mathbf{A}$	2 +	Matrix $[a_{ij}]_{i=1,\ldots,I;\,j=1,\ldots,J}$ of all observed age-classes
b_i	2 +	True age-class of fish i
$\mathbf{B}$	2 +	Vector $(b_i)_{i=1,\ldots,I}$ of true fish age-classes
c_k	1	Observed age-class of fish k ($k = 1, \ldots, K$)
$\mathbf{C}$	1	Vector $(c_k)_{k=1,\ldots,K}$ of observed fish age-classes
f_c	1	Number of observations on age-class c
d_k	1	True age-class of fish k ($k = 1, \ldots, K$)
$\mathbf{D}$	1	Vector $(d_k)_{k=1,\ldots,K}$ of true fish age-classes
		Parameters and probabilities
$\mathbf{\Phi}$	1, 2 +	Parameter vector that determines the classification matrix $\mathbf{Q}$
ϕ_m	1, 2 +	Component of $\mathbf{\Phi}$ ($m = 1, \ldots, M$)
$\mathbf{\Theta}$	1, 2 +	Complete parameter vector for a given model
θ_n	1, 2 +	Component of $\mathbf{\Theta}$ ($n = 1, \ldots, N$)
p_c^*	1, 2 +	Observed proportion of fish in age-class c
$\mathbf{P}^*$	1, 2 +	Vector $(p_c^*)_{c=1,\ldots,A}$ of observed age proportions
p_b	1, 2 +	Probability of true age b
$\mathbf{P}$	1, 2 +	Vector $(p_b)_{b=1,\ldots,A}$ of true age probabilities
$q(a\|b,\mathbf{\Phi})$	1, 2 +	Probability of reading age-class a, given the true age-class b
$\mathbf{Q}(\mathbf{\Phi})$	1, 2 +	Classification matrix $[q(a\|b,\mathbf{\Phi})]_{a-1,\ldots,A,\,b=1,\ldots,A}$

Our initial analysis requires a total of J readings for each structure, with $J > 1$. For each reading j ($j = 1, \ldots, J$), age

a_{ij} defines the age assigned to fish i ($i = 1, \ldots, I$), where a can take values from 1 to A. Although we use the term "age", our analysis treats a as an integer-valued age-class. Thus, the values $a = 1$ and $a = A$ may refer to specified minimum and maximum ages, respectively. The integer vector $\mathbf{A_i} = (a_{ij})_{j=1,\ldots,J}$ includes the ages assigned to fish i by the J readings, and the integer matrix $\mathbf{A} = [a_{ij}]_{i=1,\ldots,I;\, j=1,\ldots,J}$ represents the entire observed data set of multiple readings of all age structures.

Associated with each fish i is a true (but generally unknown) age b_i, and the integer vector $\mathbf{B} = (b_i)_{i=1,\ldots,I}$ represents the true ages of all fish in the sample. For each b, p_b denotes the probability that a fish in the sample has true age b; thus, the vector $\mathbf{P} = (p_b)_{b=1,\ldots,A}$ is subject to the constraints

$$\sum_{b=1}^{A} p_b = 1; \quad p_b \geqslant 0. \tag{1.1}$$

The number A of ages defines the length of the real vector $\mathbf{P}$. Also, entries in the integer vector $\mathbf{B}$ range from 1 to A, while the length of $\mathbf{B}$ is the number I of fish in the sample.

The relationship between observed and true fish ages is described by a classification matrix $\mathbf{Q}(\mathbf{\Phi}) = [q(a|b,\mathbf{\Phi})]_{a=1,\ldots,A;\, b=1,\ldots,A}$, which may be defined by a generic parameter vector $\mathbf{\Phi} = (\phi_m)_{m=1,\ldots,M}$. For a specified $\mathbf{\Phi}$, each matrix entry describes the probability $q(a|b,\mathbf{\Phi})$ of assigning age a to a fish, given its true age b. These probabilities are subject to the constraints

$$q(a|b,\mathbf{\Phi}) \geqslant 0 \tag{1.2}$$

$$\sum_{a=1}^{A} q(a|b,\mathbf{\Phi}) = 1 \text{ for each } b. \tag{1.3}$$

As discussed in the introduction, our analysis requires the additional assumption

$$q(b|b,\mathbf{\Phi}) > q(a|b,\mathbf{\Phi});\ a \neq b. \tag{1.4}$$

We refer to (1.4) as the "modal" assumption, since we assume that the true age is assigned with the highest (modal) probability.

Although we have represented entries in $\mathbf{Q}(\mathbf{\Phi})$ by the notation $q(a|b,\mathbf{\Phi})$, we could have used indexed notation $q_{ab}(\mathbf{\Phi})$ to emphasize the matrix character of $\mathbf{Q}(\mathbf{\Phi})$. Thus, we assume that rows and columns of $\mathbf{Q} = [q_{ab}]$ correspond to observed and true ages, respectively. Assumption (1.3) requires that columns of $\mathbf{Q}$ sum to 1, while (1.4) states that the modal probability in each column occurs along the diagonal, where $a = b$.

In practice, the dependency of $\mathbf{Q}$ on $\mathbf{\Phi}$ must be expressed analytically. We consider two possible representations for $\mathbf{Q}(\mathbf{\Phi})$. In the first we let

$$\mathbf{\Phi} = (\sigma_1, \sigma_A, \alpha) \tag{1.5}$$

and define $q(a|b,\mathbf{\Phi})$ by the sequence

$$\sigma(b) = \begin{cases} \sigma_1 + (\sigma_A - \sigma_1)\dfrac{1-e^{-\alpha(b-1)}}{1-e^{-\alpha(A-1)}}; & \alpha \neq 0 \\[2ex] \sigma_1 + (\sigma_A - \sigma_1)\dfrac{b-1}{A-1}; & \alpha = 0 \end{cases} \tag{1.6}$$

$$x_{ab}(\mathbf{\Phi}) = \frac{1}{\sqrt{2\pi}\sigma(b)} e^{-\frac{1}{2}\left[\frac{a-b}{\sigma(b)}\right]^2} \tag{1.7}$$

$$q(a|b,\mathbf{\Phi}) = \frac{x_{ab}(\mathbf{\Phi})}{\sum_{a=1}^{A} x_{ab}(\mathbf{\Phi})}. \tag{1.8}$$

In this scheme, $\sigma(b)$ in (1.6) can be loosely interpreted as the standard deviation of the observation a in (1.7). The parameters σ_1 and σ_A provide lower and upper bounds to $\sigma(b)$ for true ages $b = 1$ and $b = A$, respectively, where $\sigma_1 > 0$ and $\sigma_A > 0$. Thus, $\sigma(1) = \sigma_1$ and $\sigma(A) = \sigma_A$. The parameter α determines nonlinearity of the function $\sigma(b)$ in (1.6) (Schnute 1981), where $\sigma(b)$ becomes linear in b as α tends to 0. For a given b, the right side of (1.7) is maximized when $a = b$; thus, the mode in each column of $\mathbf{X} = [x_{ab}]$ occurs on the diagonal, consistent with the assumption (1.4) for $\mathbf{Q}$. Furthermore, the condition (1.8) guarantees that $\mathbf{Q}(\mathbf{\Phi})$ satisfies (1.3). Consequently, starting from $\mathbf{\Phi}$ in (1.5), the sequence (1.6)–(1.8) defines a matrix $\mathbf{Q}(\mathbf{\Phi})$ that conforms to the necessary criteria (1.2)–(1.4). The parameter $\sigma(b)$ differs from a true normal standard deviation in this context because we have used the density function (1.7) directly, rather than integrals of this function. We refer to (1.6)–(1.8) as a "normal model" for $\mathbf{Q}$.

Our second representation of $\mathbf{Q}(\mathbf{\Phi})$ depends on the four parameters

$$\mathbf{\Phi} = (\sigma_1, \sigma_A, \alpha, \beta). \tag{1.9}$$

In this scheme, which we call an "exponential model", $\mathbf{Q}$ is determined sequentially by (1.6),

$$x_{ab}(\mathbf{\Phi}) = \sigma(b)^{|a-b|^\beta}, \tag{1.10}$$

and (1.8), subject to the constraints

$$0 < \sigma_1 < 1, \quad 0 < \sigma_A < 1. \tag{1.11}$$

Assumptions (1.6) and (1.11) guarantee that $\sigma(b) < 1$; consequently, for each b, the right side of (1.10) is maximum when $a = b$. The extra parameter β controls the extent to which the classification matrix $\mathbf{Q}$ is dominated by its diagonal entries; as $\beta \to \infty$, $\mathbf{Q}$ becomes an identity matrix.

The concepts underlying these two representations of $\mathbf{Q}(\mathbf{\Phi})$ can readily be generalized. If $\mathbf{X} = [x_{ab}]$ is any matrix with $x_{ab} \geqslant 0$ satisfying the modal assumption, then the matrix $\mathbf{Q}$ defined by (1.8) is a classification matrix. The appropriate formulation of $\mathbf{Q}$ obviously depends on the data structure. Our parsimonious representations may be adequate for many (but not all) situations.

Our discussion so far assumes that the observed data consist of J age readings for each of I fish structures. This design is too narrow to encompass the range of practical problems associated with ageing error. We need to include the possibility that, for some structures, only individual readings are available. For example, with large samples it may be practical to obtain multiple readings for a subsample of fish and individual readings for the remainder. In this design, each of I structures is examined J times, and the remaining K structures are examined once. (If readings are performed by multiple readers, then the K structures would be assigned to readers at random.) We discuss the merits of this approach in our worked example below.

To extend our analysis to such experimental designs, we need to introduce additional notation for structures that have been examined once. Let c_k defne the age ($c = 1, \ldots, A$) assigned to fish k ($k = 1, \ldots, K$). The integer vector $\mathbf{C} = (c_k)_{k=1,\ldots,K}$ includes these observed ages. In the example above, the observed data consist of the matrix $\mathbf{A}$ of multiple age readings and the vector $\mathbf{C}$ of single age readings. We denote corresponding true ages by $\mathbf{B}$ and $\mathbf{D} = (d_k)_{k=1,\ldots,K}$, respectively, so that d_k is the true age of a fish with observed age c_k. In this context, the data $\mathbf{A}$ are analogous, but not identical, to a learning sample in a mixture problem. As we

TABLE 2. Six possible questions associated with parameters estimated from the data **A** and **C**.

1. *Age one fish*: Given the vector $\mathbf{A}_i$ for fish i and the classification matrix **Q**, what is an estimate of the true age b_i?
2. *Estimate the classification matrix and age all fish*: Given the matrix **A**, what are estimates of **Q** and **B**?
3. *Estimate the classification matrix and the true age probabilities*: Given the matrix **A**, what are estimates of **Q** and **P**?
4. *Age all additional fish*: Given the vector **C** and the classification matrix **Q**, what is an estimate of **D**?
5. *Estimate age probabilities from additional data*: Given the vector **C** and the classification matrix **Q**, what is an estimate of **P**?
6. *Estimate the classification matrix and true age probabilities from all available data*: Given the matrix **A** and the vector **C**, what are estimates of **Q** and **P**?

show below, the combination **A** and **C** can be used to estimate simultaneously the classification matrix $\mathbf{Q}(\mathbf{\Phi})$ and the probabilities **P**.

1B. Likelihood Functions

Fish age data can be used for many purposes, and appropriate analytical procedures depend on the question of interest. To focus our discussion and relate our results to the existing literature, we present a list of typical questions in Table 2. Table 3 distinguishes these questions mathematically, depending on the input data, the complete model parameter vector $\mathbf{\Theta} = (\theta_n)_{n=1,\ldots,N}$, and whether or not the classification matrix $\mathbf{Q}(\mathbf{\Phi})$ is presumed known (i.e. estimated from other data).

Question 1 asks for an estimate of the age of fish i, given **Q** and the vector $\mathbf{A}_i$ of observations. In this case, $\mathbf{\Theta} = (b_i)$, so that only one parameter is to be estimated. If ages of all fish are required, then there must be one parameter for each fish. Furthermore, if the classification matrix **Q** is also unknown, as in question 2, then an additional M parameters $\mathbf{\Phi} = (\phi_m)_{m=1,\ldots,M}$ are necessary to specify $\mathbf{Q}(\mathbf{\Phi})$. For stock assessment applications, an estimate of the vector **P** of true age probabilities is usually more important than an estimate of **B**. As clarified by questions 2 and 3, estimates of **B** and **P** involve different parameter vectors $\mathbf{\Theta}$ and correspondingly different numbers of parameters. Questions 4–6 relate to the information gained from single readings of additional structures. With **Q** given, the data **C** could be used to estimate the true age vector **D** (question 4) or the true age probabilities **P** (question 5). If all available data **A** and **C** are used, then both $\mathbf{\Phi}$ and **P** can be estimated simultaneously. Answers to questions 3 and 6 involve identical parameters, but the additional data **C** enable a larger sample size for question 6.

The estimation problems in Tables 2 and 3 can involve large numbers of parameters. In theory, $\mathbf{\Phi}$ could contain $M = A(A-1)$ parameters, since by (1.3), $A - 1$ parameters determine each column of $\mathbf{Q}(\mathbf{\Phi})$. Because **P** involves an additional $A - 1$ parameters, questions 3 and 6 could be formulated with as many as $N = A(A-1) + A - 1 = A^2 - 1$ parameters. In practice, the length of $\mathbf{\Phi}$ depends on the assumed structure of $\mathbf{Q}(\mathbf{\Phi})$. A parsimonious description, such as (1.5)–(1.8), may be more justifiable statistically.

Precise answers to the questions posed in Table 2 depend on likelihood functions (Table 4) that relate observed data to unknown parameters. The simplest likelihood [4.1] is defined by the probability that reading j results in age a_{ij} being assigned to fish i, given $\mathbf{\Phi}$ and the true age b_i. Since we assume age readings to be independent, the product of likelihoods [4.1] across readings gives the joint probability [4.2] of observing the data vector $\mathbf{A}_i$. Thus, [4.2] is the likelihood required to answer question 1. Similarly, question 2 is addressed by the product [4.3], which relates the data matrix **A** to the parameter vector $\mathbf{\Theta} = (\mathbf{\Phi},\mathbf{B})$.

Likelihoods [4.4]–[4.5] include a summation because they describe the compounded effect of two statistical processes. First, for any fish i, the true age b occurs with probability p_b. Second, given b, the vector $\mathbf{A}_i$ of observed ages occurs with probability $\Pi_{j=1}^{J} q(a_{ij}|b,\mathbf{\Phi})$. The sum over all possible true ages b in [4.4] accounts for the total probability assigned to $\mathbf{A}_i$. An additional product across fish i in [4.5] addresses question 3.

Likelihoods [4.6]–[4.10] incorporate the additional data **C** from fish aged by a single reading of an age structure. The likelihood [4.6] is analogous to [4.1] and defines the probability that age c_k is assigned to fish k, given $\mathbf{\Phi}$ and its true age d_k. The product [4.7] of probabilities [4.6] across all fish k gives the likelihood required to answer question 4. The likelihood [4.8] involves a sum over d, weighted by the probability p_d, as in [4.4]. Thus, the answer to question 5, which requires an estimate of **P**, is addressed by the product [4.9]. In general, the additional data **C** are used in conjunction with the matrix **A** of multiple age readings. The likelihood [4.10] relates both **A** and **C** to the parameters $\mathbf{\Theta} = (\mathbf{\Phi},\mathbf{P})$, as required to address question 6. Since the data **A** and **C** are independent, the joint likelihood [4.10] is formed from the product of likelihoods [4.5] and [4.9].

1C. Statistical Inference

The likelihood functions $L(\mathbf{\Theta})$ cited in Table 3 can be used to obtain inferences on the parameters $\mathbf{\Theta}$. For most purposes, it is convenient to use twice the negative log likelihood

TABLE 3. Data, number of observations, role of **Q**, parameter vector $\mathbf{\Theta}$ to be estimated, dimension N of $\mathbf{\Theta}$, and likelihood function associated with each of the six questions in Table 2. The likelihood function is referenced by the corresponding equation number in Table 4.

Question	Data	Observations	Q known?	$\mathbf{\Theta}$	N	Likelihood
1	$\mathbf{A}_i$	J	Yes	b_i	1	[4.2]
2	**A**	$I \times J$	No	$\mathbf{\Phi},\mathbf{B}$	$M + I$	[4.3]
3	**A**	$I \times J$	No	$\mathbf{\Phi},\mathbf{P}$	$M + A - 1$	[4.5]
4	**C**	K	Yes	**D**	K	[4.7]
5	**C**	K	Yes	**P**	$A - 1$	[4.9]
6	**A,C**	$I \times J + K$	No	$\mathbf{\Phi},\mathbf{P}$	$M + A - 1$	[4.10]

TABLE 4. Likelihoods associated with various estimation problems. To simplify the notation, $\mathbf{Q}(\mathbf{\Phi})$ is expressed as $\mathbf{Q}$.

[4.1] $L(a_{ij}|b_i,\mathbf{Q}) = q(a_{ij}|b_i,\mathbf{\Phi})$

[4.2] $L(\mathbf{A}_i|b_i,\mathbf{Q}) = \prod_{j=1}^{J} q(a_{ij}|b_i,\mathbf{\Phi})$

[4.3] $L(\mathbf{A}|\mathbf{B},\mathbf{Q}) = \prod_{i=1}^{I}\prod_{j=1}^{J} q(a_{ij}|b_i,\mathbf{\Phi})$

[4.4] $L(\mathbf{A}_i|\mathbf{P},\mathbf{Q}) = \sum_{b=1}^{A} p_b \prod_{j=1}^{J} q(a_{ij}|b,\mathbf{\Phi})$

[4.5] $L(\mathbf{A}|\mathbf{P},\mathbf{Q}) = \prod_{i=1}^{I}\sum_{b=1}^{A} p_b \prod_{j=1}^{J} q(a_{ij}|b,\mathbf{\Phi})$

[4.6] $L(c_k|d_k,\mathbf{Q}) = q(c_k|d_k,\mathbf{\Phi})$

[4.7] $L(\mathbf{C}|\mathbf{D},\mathbf{Q}) = \prod_{k=1}^{K} q(c_k|d_k,\mathbf{\Phi})$

[4.8] $L(c_k|\mathbf{P},\mathbf{Q}) = \sum_{d=1}^{A} q(c_k|d,\mathbf{\Phi})\, p_d$

[4.9] $L(\mathbf{C}|\mathbf{P},\mathbf{Q}) = \prod_{k=1}^{K}\sum_{d=1}^{A} q(c_k|d,\mathbf{\Phi})\, p_d$

[4.10] $L(\mathbf{A},\mathbf{C}|\mathbf{P},\mathbf{Q}) = L(\mathbf{A}|\mathbf{P},\mathbf{Q})\, L(\mathbf{C}|\mathbf{P},\mathbf{Q})$

$$(1.12)\quad l(\mathbf{\Theta}) = -2 \log L(\mathbf{\Theta})$$

which we term the “inference function”. Estimates $\hat{\mathbf{\Theta}}$ of the parameters $\mathbf{\Theta}$ are obtained by maximizing $L(\mathbf{\Theta})$, or equivalently, minimizing $l(\mathbf{\Theta})$ in (1.12). Thus, $\hat{\mathbf{\Theta}}$ is determined by

$$(1.13)\quad l(\hat{\mathbf{\Theta}}) = \min_{\mathbf{\Theta}} l(\mathbf{\Theta}).$$

Some parameter estimates may be conditional on other specified parameter values, and we indicate these with the symbol “~”. For example, let $\mathbf{\Theta} = (\mathbf{\Theta}_1, \mathbf{\Theta}_2)$, where $\mathbf{\Theta}_1$ and $\mathbf{\Theta}_2$ have dimension N_1 and N_2, respectively, with $N_1 + N_2 = N$. Given $\mathbf{\Theta}_1$, the corresponding estimate $\tilde{\mathbf{\Theta}}_2$ and minimum value $\tilde{l}$ of the inference function are defined by

$$(1.14)\quad \tilde{l}(\mathbf{\Theta}_1) = l(\mathbf{\Theta}_1, \tilde{\mathbf{\Theta}}_2(\mathbf{\Theta}_1)) = \min_{\mathbf{\Theta}_2} l(\mathbf{\Theta}_1, \mathbf{\Theta}_2).$$

In general, we use $l_i(\mathbf{\Theta})$ or $\tilde{l}_i(\mathbf{\Theta}_1)$ to denote an inference function corresponding to question i. For example,

$$(1.15)\quad l_2(\mathbf{\Theta}) = -2 \log L(\mathbf{A}|\mathbf{B},\mathbf{Q})$$

and

$$(1.16)\quad l_3(\mathbf{\Theta}) = -2 \log L(\mathbf{A}|\mathbf{P},\mathbf{Q})$$

represent the inference functions addressing questions 2 and 3, respectively.

Contours of $\tilde{l}(\mathbf{\Theta}_1)$ determine confidence regions for $\mathbf{\Theta}_1$, with $\mathbf{\Theta}_2$ considered a vector of nuisance parameters. Since twice a difference in log likelihoods is asymptotically χ^2-distributed, an approximate probability level can be associated with the boundary of a confidence region. The true value of $\mathbf{\Theta}_1$ in (1.14) lies inside the contour

$$(1.17)\quad \tilde{l}(\mathbf{\Theta}_1) = \tilde{l}(\hat{\mathbf{\Theta}}_1) + \chi^2_\omega(N_1)$$

with approximate probability ω, where $\chi^2_\omega(N_1)$ is the ω-level for the χ^2-distribution with N_1 degrees of freedom. Thus, the difference $\tilde{l}(\mathbf{\Theta}_1) - \tilde{l}(\hat{\mathbf{\Theta}}_1)$ is scaled to probability units by the χ^2-distribution.

Our analysis often depends on the special choice $\mathbf{\Theta}_1 = \mathbf{\Phi}$ in (1.14). For example, in question 1, $\tilde{b}_i(\mathbf{\Phi})$ denotes the estimated true age of fish i conditioned on $\mathbf{\Phi}$. Thus, given $\mathbf{\Phi}$, $\tilde{l}_1(\mathbf{\Phi})$ denotes the inference function minimized with respect to the true age b_i.

Uncertainty in $\hat{\mathbf{\Theta}}$ can be assessed from the estimated asymptotic covariance matrix

$$(1.18)\quad \hat{V}[\hat{\mathbf{\Theta}}] = \left[\frac{1}{2}\left(\frac{\partial^2 l(\hat{\mathbf{\Theta}})}{\partial\theta_i\, \partial\theta_j}\right)_{i=1,\ldots,N;\, j=1,\ldots,N}\right]^{-1}$$

derived from asymptotic properties of $\hat{\mathbf{\Theta}}$ in (1.13) (Kendall and Stuart 1979, p. 59–60). Estimated standard errors $\hat{\mathrm{S}}\mathrm{E}[\hat{\theta}_n]$ for the components of $\hat{\mathbf{\Theta}}$ are then given by $\sqrt{\hat{V}_{nn}}$, where $\hat{V}_{nn}$ is a diagonal component of (1.18).

Another statistical issue pertains to selecting an appropriate model structure for $\mathbf{Q}$. In the example below, we examine representations for $\mathbf{Q}(\mathbf{\Phi})$ obtained from both (1.7) and (1.10). We use the Akaike information criterion (Akaike 1974)

$$(1.19)\quad \mathrm{AIC} = l(\hat{\mathbf{\Theta}}) + 2N$$

to simplify the model identification process. A model with a low AIC value relative to other comparable models is considered to provide a good model fit. The term $2N$ in (1.19) acts as a penalty for the number of parameters; a model is favored if it can achieve a low value of $l(\hat{\mathbf{\Theta}})$ with few parameters. Akaike's (1974) method can be used for informal comparisons, where a model is a candidate for the best fit if its AIC value lies within 2 units of the minimum AIC value. More formal hypothesis tests can be based on the χ^2-distribution in (1.17).

1D. Special Cases

In the literature on stock composition models, various methods have been proposed for obtaining parameter estimates and corresponding variances. For example, Hoenig and Heisey (1987) and Kimura and Chikuni (1987) use the EM algorithm. Other authors (e.g. Pella and Robertson 1979) employ a classification-correction procedure. In their appendix, Wood et al. (1987) review some of the proposed methods and present arguments favoring the direct use of maximum likelihood. This approach requires no specialized techniques; a generic minimization algorithm is sufficient. We follow this approach in the analysis here (Appendix). Furthermore, we use straightforward numerical derivatives to evaluate $\hat{V}[\hat{\mathbf{\Theta}}]$ in (1.18).

The vectors $\mathbf{\Phi}$ and $\mathbf{P}$ are real and their estimates can be found with the aid of any search algorithm for function minimization across a real domain. By contrast, $\mathbf{B}$ and $\mathbf{D}$ are integer vectors. Their components can be estimated by evaluating the appropriate inference function l at all possible discrete values $(1, \ldots, A)$ and finding the minimum. For example, question 1 requires estimation of only one integer parameter (b_i), and this estimate can be obtained by examining the A possible values of the inference function.

Question 2 involves both real and integer parameters. To perform the necessary estimation from [4.3], first assume that $\mathbf{\Phi}$ and $\mathbf{Q} = \mathbf{Q}(\mathbf{\Phi})$ are given and use ordinary minimization to answer question 1 for each i; thus, find the estimate $\tilde{b}_i(\mathbf{Q})$ that minimizes the inference function obtained from [4.2]. Since [4.3] is a product of factors [4.2], the estimate

$$(1.20)\quad \tilde{\mathbf{B}}(\mathbf{Q}) = (\tilde{b}_i(\mathbf{Q}))_{i,\ldots,I}$$

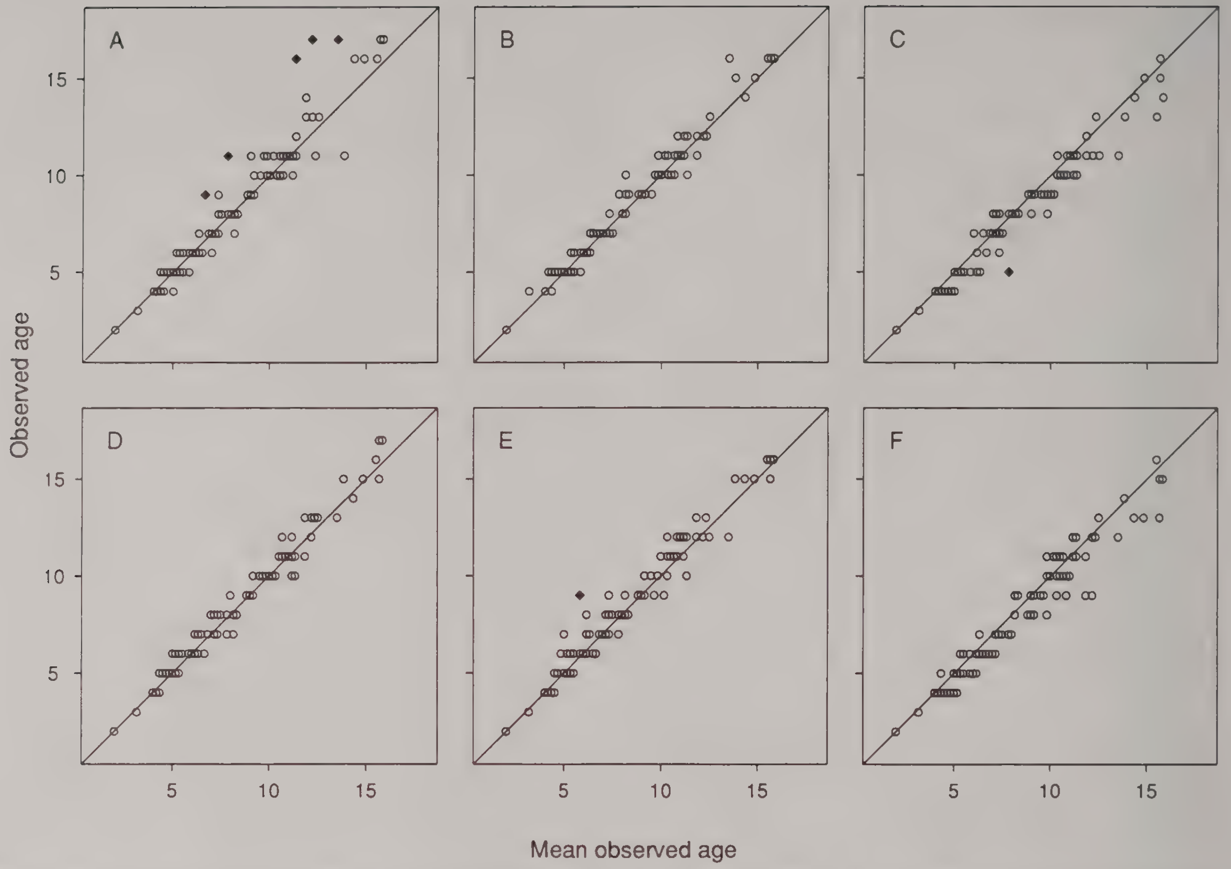

FIG. 1. Relationship between observed fish age a_{ij} and mean observed age $\frac{1}{J}\sum_{j=1}^{J} a_{ij}$ for readers A–F. A line of slope 1 is included for reference in each panel. The solid diamonds correspond to outlying points in Fig. 3.

minimizes the inference function corresponding to [4.3]. From (1.20), define the inference function

$$(1.21)\quad \tilde{l}(\mathbf{A}|\mathbf{Q}) = l(\mathbf{A}|\tilde{\mathbf{B}}(\mathbf{Q}),\mathbf{Q})$$

which depends only on $\mathbf{Q}$ and thus on the real parameter vector $\mathbf{\Phi}$. Finally, minimize $\tilde{l}(\mathbf{A}|\mathbf{Q}(\mathbf{\Phi}))$ as a function of $\mathbf{\Phi}$ to obtain the estimates $\hat{\mathbf{\Phi}}$, $\hat{\mathbf{Q}} = \mathbf{Q}(\hat{\mathbf{\Phi}})$, and $\hat{\mathbf{B}} = \tilde{\mathbf{B}}(\hat{\mathbf{Q}})$. These estimates resolve question 2.

The parameters $\mathbf{\Phi}$ and $\mathbf{P}$ of question 3 are real. However, estimation of $\hat{\mathbf{P}}$ can be difficult if one or more (unknown) components of $\mathbf{P}$ are 0. To ensure that the constraints (1.1) are met during minimization, we use surrogate parameters z_b, defined such that

$$(1.22)\quad p_b = \frac{1}{2}(1 - \cos \pi z_b).$$

Thus, values z_b in the range (0,1) or, more generally, in the range $(-\infty, \infty)$ correspond to values p_b in the range (0,1). We choose an age b_0 for which p_{b_0} appears relatively large, use (1.22) for $b \neq b_0$, and then compute

$$(1.23)\quad p_{b_0} = 1 - \sum_{b \neq b_0} p_b.$$

Thus, the parameter vector $\mathbf{P}$ is determined by $A - 1$ surrogate parameters z_b with $b \neq b_0$.

Question 4 requires estimating the integer vector $\mathbf{D}$, given $\mathbf{Q}$ and $\mathbf{C}$. In this case, there is precisely one parameter d_k for each observation c_k, and the estimate $\hat{d}_k$ maximizes $q(c_k|d)$ as a function of d. Thus, the estimate can be obtained by inspecting row c_k of the matrix $\mathbf{Q}$. Incidentally, the modal hypothesis (1.4) does not imply that $\hat{d}_k = c_k$, because (1.4) asserts only that the column maximum occurs on the diagonal of $\mathbf{Q}$.

For question 5, the estimates $\hat{\mathbf{P}}$ sometimes have a simple analytical representation. In general, the number K of additional fish is much greater than the number A of ages. If f_c represents the frequency of observing age c, then the observed proportion of age c fish is given by the ratio

$$(1.24)\quad p_c^* = \frac{f_c}{\sum_{c=1}^{A} f_c}.$$

The observed proportions (1.24) define the vector $\mathbf{P}^* = (p_c^*)_{c=1,\ldots,A}$. The likelihood

$$(1.25)\quad L(\mathbf{C}|\mathbf{P},\mathbf{Q}) = \prod_{c=1}^{A}\left[\sum_{d=1}^{A} q(c|d,\mathbf{\Phi})\, p_d\right]^{f_c}$$

is equivalent to [4.9] and accounts for the grouping of observations. A simple proof (Wood et al. 1987, appendix, proposition 1) based on (1.25) gives

TABLE 5. Estimates of the parameters $\mathbf{\Phi} = (\sigma_1, \sigma_A, \alpha, \beta, \gamma)$ for questions 2 and 3, based on the normal (Norm), exponential (Exp), and reader effects (Rdr) models. The corresponding AIC and minimum values of the inference function are also given for comparison. Different cases represent the full models and models reduced by the constraints $\alpha = 0$, $\beta = 1$, or $\gamma = 1$. Values b are listed for which estimates $\hat{p}_b = 0$.

Case	Question	Model	N	$\hat{\sigma}_A$	$\hat{\sigma}_1$	$\hat{\alpha}$	$\hat{\beta}$	$\hat{\gamma}$	b with $\hat{p}_b = 0$	$l(\hat{\mathbf{\Theta}})$	AIC
1	2	Norm	127	0.495	2.038	−0.166	—	—	—	1645.8	1899.8
2	2	Norm	126	0.369	1.411	0	—	—	—	1650.6	1902.6
3	2	Exp	128	0.080	0.534	−0.012	1.204	—	—	1600.7	1856.7
4	2	Exp	127	0.074	0.523	0	1.204	—	—	1600.7	1854.7
5	2	Exp	127	0.088	0.461	−0.046	1	—	—	1607.3	1861.3
6	3	Norm	18	0.458	1.858	−0.109	—	—	13 15 17	2189.8	2225.8
7	3	Norm	17	0.366	1.472	0	—	—	13 15 17	2191.7	2225.7
8	3	Exp	19	0.101	0.604	−0.061	1.187	—	17	2141.4	2179.4
9	3	Exp	18	0.072	0.537	0	1.184	—	17	2141.7	2177.7
10	3	Exp	18	0.095	0.477	−0.052	1	—	17	2147.0	2183.0
11	3	Rdr	25	0.046	0.352	−0.130	0.849	0.704	13 17	2014.3	2064.3
12	3	Rdr	24	0.031	0.252	0	0.851	0.682	13 17	2015.9	2063.9
13	3	Rdr	24	0.044	0.380	−0.131	0.869	1	13 17	2015.2	2063.2
14	3	Rdr	23	0.028	0.271	0	0.864	1	13 17	2017.1	2063.1

$$(1.26) \quad \hat{\mathbf{P}} = \mathbf{Q}^{-1}\mathbf{P}^*$$

if the conditions (1.1) are satisfied by $\hat{\mathbf{P}}$. Equation (1.26) was discussed in general terms in the introduction. The inverse classification matrix $\mathbf{Q}^{-1}$ acts on the observed proportions $\mathbf{P}^*$ to reverse the smoothing effect of ageing error and thus to give the estimate $\hat{\mathbf{P}}$.

2. Pollock Example

2A. Data Description

The walleye pollock data were obtained from a reference sample prepared by the Alaska Fisheries Science Center (National Marine Fisheries Service, Seattle, WA). The sample contained 125 otolith pairs from the Gulf of Alaska and four areas of the Bering Sea, collected between October 1984 and February 1985. Each otolith pair was independently examined by six experienced readers from three countries using the break and burn method (Chilton and Beamish 1982).

Our analysis requires a value for the maximum age A. For the pollock data, assigned ages for one fish ranged from 21 to 24 yr and the next oldest assigned age was 17 yr. We excluded data on this fish to avoid extrapolation for missing ages. Thus, we set $A = 17$. Although data from old fish may be important for biological interpretation, our purpose here is illustration of the basic statistical methods. We seek estimates of the classification matrix $\mathbf{Q}(\mathbf{\Phi})$ within an age range where multiple readings are available.

We examined the remaining data by comparing ages assigned by each reader to the mean assigned age (Fig. 1). Readers A and E tend to overestimate and readers C and F underestimate ages relative to the mean. A more complex model, with extra parameters to accommodate reader effects, is presented in section 3. Here, we treat reader effects as part of the overall model variance. Since there are no large consistent deviations by any one reader, the data are adequate to address questions 2 and 3. Observed ages range from 2 to 17 yr, with the variance in assigned age tending to increase with the mean. There is complete agreement on the three fish assigned an age of 2 yr. Following the notation in Table 1, the data consist of $I' = 124$ fish, with $J = 6$ readings of each age structure. The formal notation (Table 1) would use $a = 1, \ldots, 16$ to reference the 16 age-classes. For clarity, however, we let the symbol a denote an observed age $a = 2, \ldots, 17$.

2B. Question 2 Analysis

Technical details of model application are deferred to the Appendix. Parameter estimates

$$(2.1) \quad \hat{\mathbf{\Phi}} = (\hat{\sigma}_1, \hat{\sigma}_A, \hat{\alpha}, \hat{\beta})$$

for question 2 are provided in Table 5, cases 1–5, along with values of $l_2(\hat{\mathbf{\Theta}})$ and the AIC. These five cases differ in the model used for $\mathbf{Q}$ and constraints imposed on the parameters $\mathbf{\Phi}$. For the normal model from (1.7), the full parameter vector (1.5) is included in case 1 whereas α is constrained to 0 in case 2. For the exponential model from (1.10), we consider the full parameter vector (1.9) (case 3) and parameter vectors reduced by the constraints $\alpha = 0$ (case 4) and $\beta = 1$ (case 5). Based on AIC values from (1.19), cases 3 and 4 are the most plausible candidates for the pollock data; AIC values for other cases are more than 6 units above the minimum. Cases 3 and 4 employ the exponential model and they can be compared by formal hypothesis tests using (1.17). It is not possible to reject the hypothesis that $\alpha = 0$, since minimum inference values $l_2(\hat{\mathbf{\Theta}})$ for cases 3 and 4 agree to the reported number of decimal places. Thus, case 4 provides the best model fit for question 2.

The success of the exponential model is best understood by comparing the estimates $\mathbf{Q}(\hat{\mathbf{\Phi}})$ for normal and exponential models. Estimated probability distributions $q(a|b, \hat{\mathbf{\Phi}})$ for case 4 are more peaked than the corresponding distributions for case 1 (Fig. 2), declining rapidly from the mode at $a = b$. Thus, the exponential model leads to relatively leptokurtotic distributions. For most fish, however, the rounded mean observed age, the modal observed age, and the estimated true age $\hat{b}_i$ all agree. Estimates $\hat{\mathbf{B}}$ differ between cases 1 and 4 for only 8 (6%) of the 124 fish. For these fish, the exponential model tends to select the most frequent observation a whereas the normal model is more sensitive to outliers.

Because inference functions in this paper are not expressed as sums of squares, residual analysis in the usual sense is not possible. Nevertheless, the inference function (1.15) for question 2 is computed as a sum of positive terms, where each is the negative log of a probability. To investigate the weight attached to each observation, we define the inference contribution

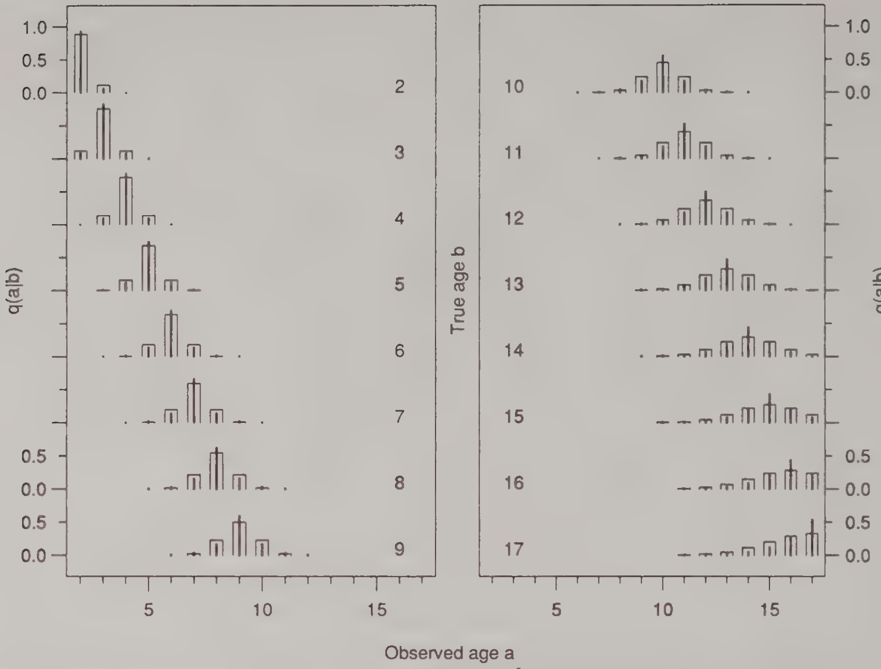

FIG. 2. Relationship between estimated probability $q(a|b,\hat{\Phi})$ and observed age a, given the specified true age b. Open bars represent the normal model (Table 5, case 1) for question 2 and centered vertical lines represent the exponential model (case 4).

$$(2.2) \qquad \epsilon_{ij} = -2 \log q(a_{ij}|\hat{b}_i, \hat{\Phi})$$

from a single term of the inference sum. Figure 3 shows that the contributions ϵ_{ij} increase approximately linearly with the discrepancy

$$(2.3) \qquad \delta_{ij} = |a_{ij} - \hat{b}_i|$$

between observed and estimated age-classes. For the pollock data, the discrepancy δ_{ij} takes integer values between 0 and 6; however, values δ_{ij} in Fig. 3 are jittered to improve the data display. Gaps in the frequency distribution of the contributions arise because ϵ_{ij} in (2.2) can admit only certain values. There are seven observations in Fig. 3 from six fish with contributions $\epsilon_{ij} > 10$. These observations are identified in Fig. 1; five are due to reader A, one to reader C, and one to reader E. Clearly, inconsistencies among readers inflate the inference function (1.15) and increase the uncertainty in $\hat{\mathbf{B}}$.

2C. Question 3 Analysis

As described in the introduction, most ageing error analyses aim to determine true age proportions **P**. Thus, question 3 has greater practical importance than question 2. For the pollock data, the results for question 3 (Table 5) are similar to those obtained for question 2. The exponential model leads to much lower AIC values than the normal model. Case 9 provides the best overall fit; again, we cannot reject the hypothesis that $\alpha = 0$.

Estimates $\hat{\mathbf{P}}$ vary little with final model choice among cases 6–10. Table 6 illustrates these results for cases 8–10, which are based on the exponential model. Differences among these cases relate to the assumed structure of **Q**. Table 7 provides a means of comparing estimates $\hat{\mathbf{Q}}$, where for each age b, the variance $V[a|b]$ can be calculated directly from the corresponding column of $\hat{\mathbf{Q}}$. Variances $V[a|b]$ for cases 8–10 are similar at young ages, but case 9 has a lower variance for older ages. Thus, flexibility in the parameter α allows for larger ageing error at these ages. Our inability to reject the hypothesis that $\alpha = 0$ reflects our small sample size at old ages. This observation has implications for sample design. Sample sizes must be large enough to estimate the distribution $q(a|b)$ for each age b. Typically, ageing error is larger and fewer age structures are available at older ages. Thus, it may be desirable to increase the number of readings of older age structures.

Small sample sizes at older ages also complicate the estimate $\hat{\mathbf{P}}$. In a typical fisheries data set, the exact dimension of **P** is poorly determined; the observed maximum age A is a random variable dependent on small values p_b for large b. For the normal model for **Q** (cases 6–7), we obtain the estimates $\hat{p}_{13} = \hat{p}_{15} = \hat{p}_{17} = 0$. Similarly, for the exponential model for **Q** (cases 8–10), we obtain the estimate $\hat{p}_{17} = 0$. Thus, maximum likelihood estimates for these cases lie on the parameter boundary defined by the constraint (1.1). Nevertheless, all values p_b ($b = 2, \ldots, 17$) are free parameters in the calculation, and we determine N and the AIC accordingly. In essence, our analysis suggests that the proportion of fish older than age 12 is small and poorly determined. A more rigorous analysis might use the new parameter

$$(2.4) \qquad p_+ = \sum_{b=13}^{17} p_b$$

in place of p_{13}, and treat $p_{14}, \ldots, p_{17}$ as nuisance parameters. Although this would circumvent problems associated with the random behaviour of A, it would make the analysis much more complicated technically. We prefer the less formal approach here, with the understanding that information on proportions p_b with $b \geqslant 13$ is really limited to the composite parameter p_+ in (2.4). Estimates $\hat{p}_+$ are indeed similar among cases 8–10 (Table 6).

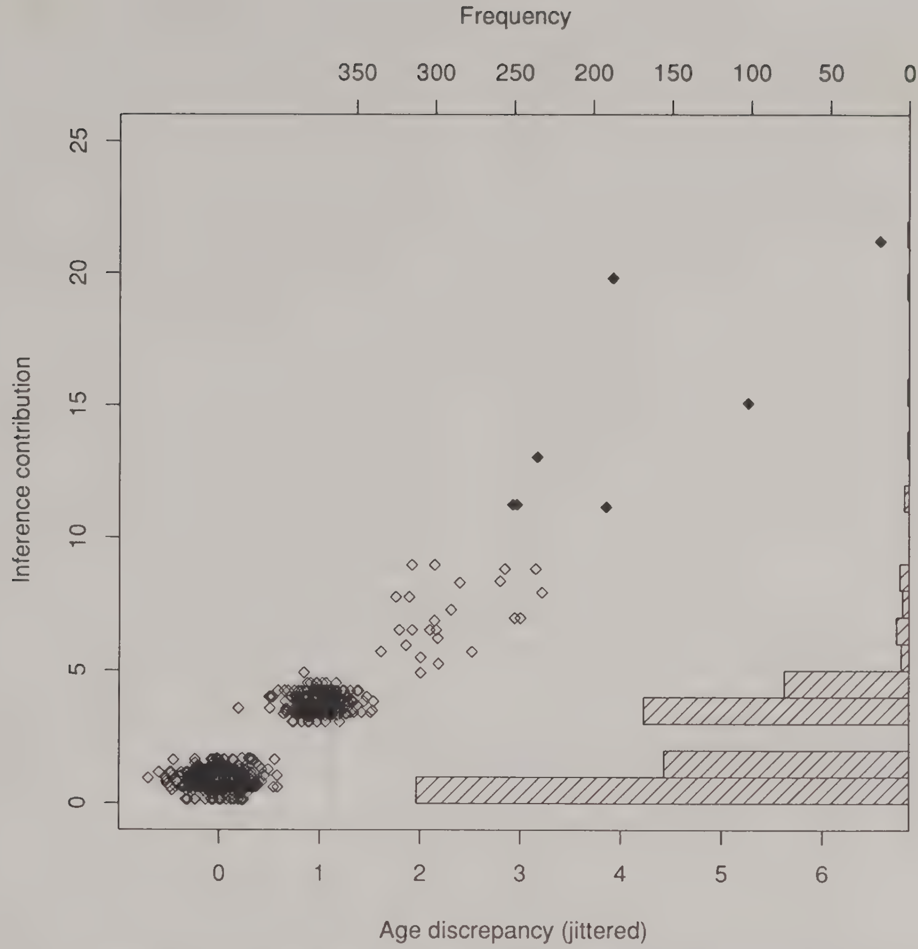

FIG. 3. Relationship between inference contributions ϵ_{ij} in (2.2) and the age discrepancy δ_{ij} in (2.3), based on the model in Table 5, case 4. The quantity δ_{ij} is integer-valued, but has been jittered to better display the data. Solid diamonds indicate values $\epsilon_{ij} > 10$. Horizontal bars reflect the frequency distribution of ϵ_{ij} values.

An informal residual analysis for question 3 can be conducted as for question 2. The positive inference contribution for one fish,

$$(2.5) \qquad \zeta_i = -2 \log \left[\sum_{b=1}^{A} \hat{p}_b \prod_{j=1}^{J} q(a_{ij}|b,\hat{\Phi}) \right]$$

increases approximately linearly with

$$(2.6) \qquad \eta_i = \sum_{j \neq j'} |a_{ij} - a_{ij'}|$$

a measure of disparity among readings for fish i (Fig. 4). Since a_{ij} and b are integers, the quantity ζ_i in (2.5) admits only certain values, and gaps are apparent in the distribution of (2.5). A few fish contribute large values (2.5) and thus have high leverage in the inference function. The four fish with values $\zeta_i > 30$ also contribute to outlying points in Fig. 1 and 3.

Approximate confidence intervals for $\hat{p}_b$ can be obtained from $\hat{\mathrm{SE}}(\hat{p}_b)$, determined by the asymptotic covariance matrix in (1.18), or from the relationship (1.17) involving the χ^2-distribution. The two methods lead to 95% intervals of similar width for each value b (Table 8), except where the lower bound lies on the constraints $p_b = 0$. Likelihood intervals from (1.17) are located asymmetrically around the estimates $\hat{p}_b$; midpoints of the interval from (1.18) lie well above the corresponding estimates $\hat{p}_b$. Such asymmetries are intuitively appealing because symmetric intervals defined by $\hat{\mathrm{SE}}(\hat{p}_b)$ would have negative lower bounds in the cases $b = 3$ and $b = 14$. Estimates $\hat{p}_b$ are assumed to be unconstrained in (1.18) and we cannot obtain meaningful measures $\hat{\mathrm{SE}}(\hat{p}_b)$ at the boundary of the [0,1] range for p_b. For example, we cannot use (1.18) to obtain $\hat{\mathrm{SE}}(\hat{p}_{17})$. Nevertheless, because of computational intensity associated with (1.17) (Appendix), we resort to (1.18) as our measure of uncertainty.

If ages were known perfectly, the expected variance of $\hat{\mathbf{P}}$ could be estimated directly from the multinomial distribution, where the multinomial variance estimate $\hat{p}_b(1 - \hat{p}_b)/I$ depends on the number of fish I in the sample. Because ageing error introduces additional uncertainty, the estimated variance $\hat{V}[\hat{p}_b]$ from (1.18) is generally larger than the corresponding multinomial variance. Thus, a larger sample size is required to estimate $\hat{p}_b$ with a specified precision when ageing error is present than when exact ages are known. The relationship between $\hat{V}[\hat{p}_b]$ and $\hat{p}_b(1 - \hat{p}_b)$ is approximately linear, with a slope near

TABLE 6. Percentages corresponding to the estimates $\hat{\mathbf{P}}$ for cases 8–14 in Table 5. The estimate of p_+ in (2.4) is also given for comparison.

	$100 \times \hat{p}_b$						
b	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Case 14
2	2.4	2.4	2.4	2.4	2.4	2.4	2.4
3	0.8	0.8	0.8	0.8	0.8	0.8	0.8
4	3.9	3.8	3.9	3.4	3.4	3.4	3.3
5	23.8	23.8	23.7	24.3	24.3	24.3	24.4
6	5.7	5.7	5.7	5.9	5.8	5.9	5.8
7	19.2	19.2	19.4	19.3	19.3	19.3	19.3
8	5.4	5.4	5.2	5.1	5.1	5.1	5.1
9	6.3	6.3	6.3	6.2	6.3	6.3	6.3
10	9.0	9.1	9.3	9.9	10.0	9.9	9.9
11	12.4	12.4	11.9	10.3	10.3	10.4	10.5
12	4.3	4.3	4.2	6.7	6.7	6.6	6.6
13	0.9	1.0	1.3	0.0	0.0	0.0	0.0
14	0.9	1.0	0.7	1.1	1.2	1.0	1.1
15	1.8	1.7	2.0	1.4	1.3	1.5	1.4
16	3.1	3.1	3.0	3.2	3.2	3.2	3.2
17	0.0	0.0	0.0	0.0	0.0	0.0	0.0
+	6.8	6.8	7.0	5.7	5.7	5.7	5.7

$1/I$ (Fig. 5). The estimated variance for ages 2 and 3 conforms very nearly to the multinomial prediction because these ages are measured with little error. Thus, when $b = 2$ or $b = 3$, $q(a|b)$ is nearly 1 when $a = b$ and 0 otherwise. For $b > 3$, $\hat{V}[\hat{p}_b]$ exceeds the multinomial prediction, where large deviations for ages 10–12 correspond to high levels of ageing error. The small deviation for age 16 is an artifact of our analysis, due to the fact that $\hat{\mathbf{P}}$ lies on the constraint $p_{17} = 0$. Thus, if age 17 is deemed impossible, then increased certainty is attached to a reading $a_{ij} = 16$.

In summary, this analysis of variance in $\hat{\mathbf{P}}$ shows that precision depends on the number I of fish age structures, rather than the total number of readings IJ. Multiple readings of individual structures are informative for $\mathbf{Q}$, but not for $\mathbf{P}$. Furthermore, as ageing error increases, data from individual age structures become less and less informative for $\mathbf{P}$. For this reason, we emphasize that sample sizes must be increased with increasing ageing error to yield a specified precision in $\hat{\mathbf{P}}$.

As discussed in the introduction, the true age proportions might be estimated naively from the frequency distribution of the estimates $\hat{\mathbf{B}}$ for question 2. Let $\mathbf{P}' = (p'_b)_{b=1,\ldots,A}$ represent age proportions determined from $\hat{\mathbf{B}}$. We have stated earlier that ageing error tends to smooth the observations $\mathbf{P}^*$ and that age proportions $\mathbf{P}'$ do not fully account for the smoothing effect. This is because of uncertainty in $\hat{\mathbf{B}}$. As J increases, $\hat{\mathbf{B}}$ asymptotically approaches its true value under the modal hypothesis (1.4). However, J will be small for most applications, leading to bias in the calculation of $\mathbf{P}'$. Evidence of smoothing in the pollock data is illustrated in Fig. 6. For the two dominant ages $b = 5$ and $b = 7$, $p^*_b < p'_b < \hat{p}_b$ as expected. Similarly, $p^*_b > p'_b > \hat{p}_b$ for ages 4, 6, and 8, adjacent to the dominant ages. Thus, the distributions defined by the observations $\mathbf{P}^*$, the naive estimates $\mathbf{P}'$, and the likelihood estimates $\hat{\mathbf{P}}$ become progressively more peaked.

For the pollock data, we are unable to distinguish statistically among $\mathbf{P}^*$, $\mathbf{P}'$, and $\hat{\mathbf{P}}$. For example, each observation p^*_b lies within $2 \times \hat{\text{SE}}(\hat{p}_b)$, and the inference function $l_3(\hat{\boldsymbol{\Theta}})$ is relatively insensitive to small variations in $\hat{p}_b$. Differences among $\mathbf{P}^*$, $\mathbf{P}'$, and $\hat{\mathbf{P}}$ are still biologically meaningful, however; small deviations in $\hat{\mathbf{P}}$ can have a large impact on any subsequent analyses. Our inability to reject the observations $\mathbf{P}^*$ as estimates of $\hat{\mathbf{P}}$ simply reflects our small sample of 124 fish. The appropriate choice of sample size is an obvious subject for future work.

3. Reader Effects

In section 1, we described general analytical methods needed to estimate ageing error from a sample with multiple age readings. Here, we suggest how the methods might be tailored to a specific data set. We present a more complex model for the pollock data, acknowledging two features of these data not considered above. First, the J readings of each age structure were conducted by the same J readers. Thus, we can examine the data for possible reader effects. Second, the readers agreed completely on the age of the youngest fish. Our revised model acknowledges this by assuming that no ageing error is associated with ages 1 to A_1.

The extended pollock model depends on the $5 + J$ parameters

$$(3.1) \qquad \boldsymbol{\Phi} = (\sigma_1, \sigma_A, \alpha, \beta, \gamma, r_1, \ldots, r_J)$$

subject to the constraints

$$(3.2) \qquad \sum_{j=1}^{J} r_j = 0$$

where r_j represents the relative bias of reader j at age A and γ determines the change in bias with age. Based on the reader effects in (3.1), the revised classification matrix must be indexed by reader. We define the classification matrix $\mathbf{Q}_j$ by the sequence

$$(3.3) \qquad \sigma(b) = \begin{cases} \sigma_1 + (\sigma_A - \sigma_1)\dfrac{1-e^{-\alpha(b-A_1-1)}}{1-e^{-\alpha(A-A_1-1)}}; & \\ \qquad\qquad \alpha \neq 0, b > A_1 & \\ \sigma_1 + (\sigma_A - \sigma_1)\dfrac{b-A_1-1}{A-A_1-1}; & \\ \qquad\qquad \alpha = 0, b > A_1 & \end{cases}$$

$$(3.4) \qquad w(b) = \gamma + (1-\gamma)\frac{b - A_1 - 1}{A - A_1 - 1}$$

$$(3.5) \qquad x_{abj}(\boldsymbol{\Phi}) = \sigma(b)^{|a-b-w(b)r_j|^\beta}; \quad \alpha > A_1, b > A_1$$

$$(3.6) \qquad q_j(a|b, \boldsymbol{\Phi}) = \frac{x_{abj}(\boldsymbol{\Phi})}{\sum\limits_{a=A_1+1}^{A} x_{abj}(\boldsymbol{\Phi})}; \quad \alpha > A_1, b > A_1$$

$$(3.7) \qquad \mathbf{Q}_j = \begin{pmatrix} \mathbf{I} & \mathbf{0} \\ \mathbf{0} & \mathbf{Q}'_j \end{pmatrix}$$

where we assume that ages 1 to A_1 can be determined without error. Thus, the classification matrix $\mathbf{Q}_j$ in (3.7) is composed of four submatrices. The $A_1 \times A_1$ identity matrix $\mathbf{I}$ accounts for the youngest A_1 ages. Two rectangular null matrices $\mathbf{0}$ ensure that $q_j(a|b,\boldsymbol{\Phi}) = 0$ for $a \neq b$ when $b \leqslant A_1$ or $a \leqslant A_1$. The $(A - A_1) \times (A - A_1)$ matrix $\mathbf{Q}'_j$ describes ageing error associated with the oldest $A - A_1$ ages.

Equations (3.3)–(3.6), defining elements $q_j(a|b,\boldsymbol{\Phi})$ of $\mathbf{Q}'_j$, are analogous to (1.6)–(1.10) earlier. The modification (3.3) simply allows $\sigma_1 = \sigma(A_1 + 1)$ to represent the first age where ageing error is present. Reader effects enter in equation (3.5), where $w(b)r_j$ acts as an offset in the definition of $x_{abj}(\boldsymbol{\Phi})$ for a given b and j. We assume that reader effects vary linearly with age, dependent on the weight function $w(b)$ in (3.4). From (3.4), the offset $w(b)r_j$ takes the values γr_j and r_j when $b = A_1 + 1$ and $b = A$, respectively. Thus, the parameter r_j describes reader effects at the oldest age and the parameter γ describes the reduction in r_j at age $A_1 + 1$. Because $\sigma(b)$ and β are independent of j, reader effects result in only minor changes to the shape of the distribution $q_j(a|b,\mathbf{\Phi})$ for a given b. These changes are due to the mode shift in (3.5), combined with the normalization (3.6).

The likelihood function to address question 3 is given by

$$(3.8)\quad L(\mathbf{A}|\mathbf{P}, \mathbf{Q}_j) = \prod_{i=1}^{I} \sum_{b=1}^{A} p_b \prod_{j=1}^{J} q_j(a_{ij}|b,\mathbf{\Phi}),$$

a modification of [4.5]. Parameter estimates obtained from the inference function corresponding to (3.8) are listed in Table 5 (cases 11–14). We considered the full model as well as models reduced by the constraints $\alpha = 0$ and $\gamma = 1$. Cases 11–14 agree as to the estimates $\hat{p}_{13} = 0$ and $\hat{p}_{17} = 0$ and have similar AIC values. There is a large decrease in AIC values between cases 8–10 and 11–14. Obviously, reader effects contribute significantly to model fit. The estimates $\hat{\sigma}_1$ and $\hat{\sigma}_A$ are also smaller for cases 11–14, although strict comparisons with cases 8–10 are complicated by a corresponding change in $\hat{\beta}$. Furthermore, σ_1 describes age 2 for cases 8–10 and age 3 for cases 11–14 because of the modification (3.3). The reader effects are small in magnitude (Table 9). With $\gamma = 1$ (cases 13–14), each estimate $\hat{r}_j$ represents a maximum offset in (3.5) of less than 0.5 yr. Estimates are slightly larger in cases 11–12, where the estimates $\hat{\gamma} < 1$ indicate that age-dependent effects $w(b)r_j$ increase with b.

An ageing error analysis is typically conducted to obtain estimates $\hat{\mathbf{P}}$. These estimates are relatively unaffected by the inclusion of reader effects (Table 6). Differences are minor between most corresponding values $\hat{p}_b$ for cases 8–14. The largest differences between the exponential and reader effects models occur for ages 11–12, the ages with the largest deviations in $\hat{V}[\hat{p}_b]$ from the expected multinomial variance in Fig. 5. Reader bias for age 12 is clearly evident from Fig. 1. Readers A and C each assigned age 12 to one structure whereas readers B and E assigned age 12 to 10 and 13 structures, respectively.

Variances $V[a|b]$ for cases 11–14 cannot be calculated directly as in cases 8–10. The exponential model in section 2 involves a single classification matrix $\mathbf{Q}$, while the model here depends on J distinct matrices $\mathbf{Q}_j$. To relate this model to the earlier one, suppose that (for a given b) the random variable a results from compounding two processes. First, reader j is selected at random and then a reading is obtained from the error distribution $q_j(a|b)$ for that reader. The resulting variance of $a|b$ can be expressed as the sum

$$(3.9)\quad V[a|b] = E_j[V_a[a|b, j]] + V_j[E_a[a|b, j]]$$

where (3.9) is a general result for such compound distributions. Here, $V_a[a|b, j]$ measures the variability of a single reader and the expectation E_j averages this within-reader variabiilty. Similarly, $E_a[a|b, j] - b$ measures the bias of a single reader, and the variance of this quantity (the same as the variance of $E_a[a|b, j]$) measures bias variability among readers. Thus, (3.9) both provides a means of computing $V[a|b]$ for the reader effects model and partitions this variance into components within and among readers.

Variances $V[a|b]$ are similar for exponential and reader effects models, tending to increase with b (Table 7). Higher values $V[a|b]$ are associated with cases 8, 10, 11, and 13, for which α is unconstrained. The partitioning of $V[a|b]$ into components within and among readers is illustrated for case 11 only, but comparable results apply to other cases. The ability to partition this variance accounts for the drop in AIC values between cases 8–10 and cases 11–14. However, the magnitude of $V[a|b]$ can be attributed mainly to within-reader variability. The small among-reader variability tends to increase with b, consistent with our assumption of age-dependent reader effects in cases 11–12. Our general conclusion for this analysis is that reader variability can be partitioned, but not reduced, by extending the exponential model to include reader effects. Because corrections to age proportions stem from the composite ageing error, estimates $\hat{\mathbf{P}}$ (Table 6) are similar between exponential and reader effects models.

The model (3.1)–(3.7) is a simple implementation of reader effects, consistent with the modal hypothesis (1.4). We have

TABLE 7. Calculated variances $V[a|b]$ for cases 8–14 in Table 5. For case 11, the components $E_a[V_j[a|b, j]]$ and $V_j[E_a[a|b, j]]$ of (3.9) are also given, indicated by the columns V_jE_a and E_aV_j, respectively.

	Case 8	Case 9	Case 10	Case 11			Case 12	Case 13	Case 14
b	$V[a\|b]$	$V[a\|b]$	$V[a\|b]$	E_aV_j	V_jE_a	$V[a\|b]$	$V[a\|b]$	$V[a\|b]$	$V[a\|b]$
2	0.10	0.07	0.12	0.00	0.00	0.00	0.00	0.00	0.00
3	0.22	0.19	0.23	0.14	0.01	0.15	0.11	0.16	0.12
4	0.30	0.28	0.33	0.27	0.04	0.31	0.28	0.33	0.30
5	0.37	0.36	0.41	0.35	0.05	0.40	0.39	0.41	0.40
6	0.44	0.44	0.49	0.41	0.06	0.47	0.49	0.47	0.49
7	0.52	0.54	0.58	0.47	0.07	0.54	0.58	0.54	0.58
8	0.61	0.64	0.69	0.55	0.08	0.63	0.68	0.62	0.67
9	0.72	0.76	0.81	0.64	0.08	0.72	0.78	0.71	0.77
10	0.86	0.89	0.96	0.75	0.09	0.84	0.88	0.82	0.87
11	1.02	1.05	1.13	0.88	0.10	0.98	1.00	0.97	0.98
12	1.22	1.21	1.32	1.04	0.11	1.14	1.11	1.14	1.10
13	1.43	1.38	1.50	1.20	0.11	1.32	1.20	1.32	1.19
14	1.61	1.49	1.62	1.35	0.11	1.46	1.25	1.49	1.24
15	1.69	1.49	1.66	1.44	0.10	1.55	1.22	1.59	1.21
16	1.68	1.37	1.64	1.51	0.08	1.59	1.10	1.68	1.10
17	1.80	1.27	1.75	1.74	0.03	1.77	0.94	1.96	0.97

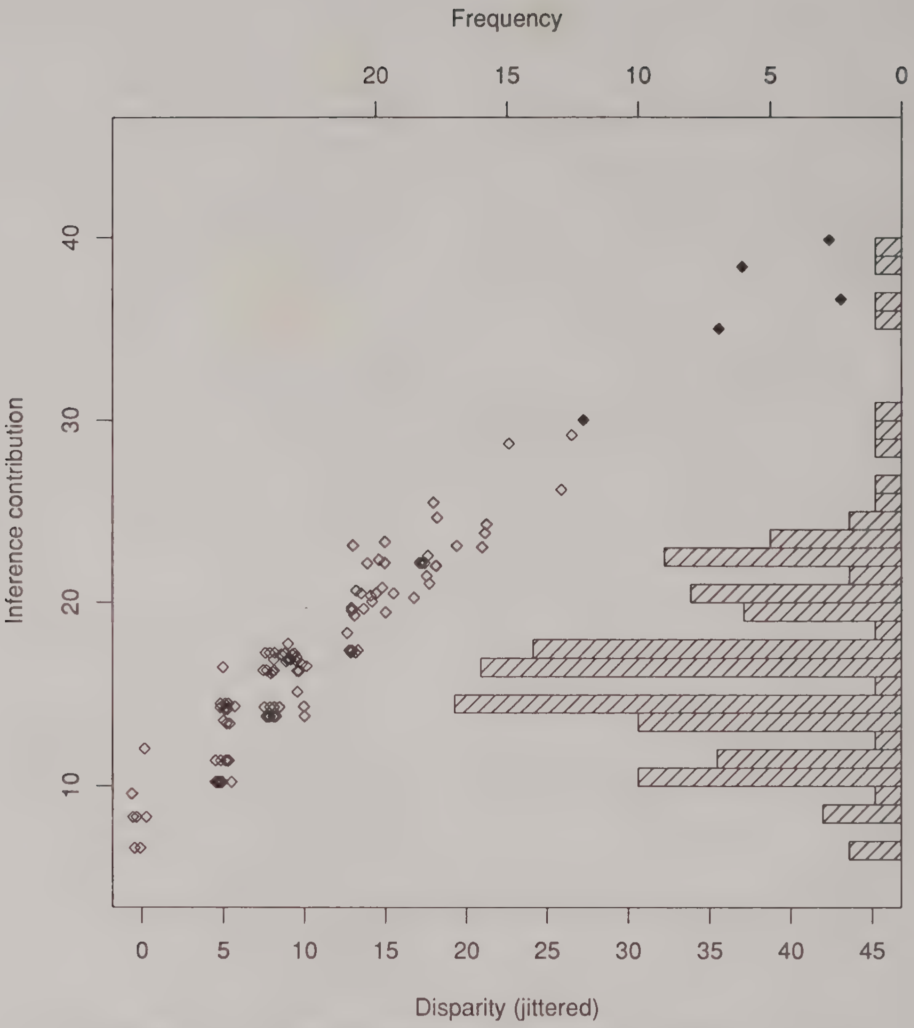

FIG. 4. Relationship between inference contributions ζ_i in (2.5) and the disparities η_i in (2.6), where η_i is calculated from the model in Table 5, case 9. The quantity η_i can take only certain integer values (0, 5, 6, 8, . . .), but has been jittered to better display the data. Solid diamonds represent the five fish identified as outliers in Fig. 1 and 3. Horizontal bars reflect the frequency distribution of ζ_i values.

TABLE 8. For selected ages b from case 9 in Table 5: estimates $\hat{p}_b$, widths $2 \times 1.96 \times \hat{SE}(\hat{p}_b)$ of 95% confidence intervals obtained from the asymptotic covariance matrix (1.18), 95% confidence intervals from the χ^2-distribution in (1.17), and corresponding midpoints and widths of the latter intervals. For $b = 14$, the left endpoint of the 95% interval is constrained at $p_b = 0$.

b	$\hat{p}_b$	$2\times1.96\times\hat{SE}(\hat{p}_b)$	95% interval	Midpoint	Width
3	0.008	0.031	(0.000,0.035)	0.018	0.035
4	0.038	0.073	(0.012,0.085)	0.048	0.073
11	0.124	0.137	(0.065,0.200)	0.133	0.136
14	0.010	0.058	(0.000,0.050)	0.025	0.050

assumed that the shape of the distribution $q_j(a|b,\Phi)$ for a given b is similar for each reader, with the distribution mode shifted according to the relative bias $w(b)r_j$. More complex formulations could be implemented. For example, $w(b)$ could depend nonlinearly on b, and the parameters σ_1, σ_2, α, and β could also be indexed by reader.

4. Concluding Remarks

We have presented the principal analytical methods needed to estimate ageing error from a sample with multiple age readings. One possible model extension incorporating reader effects was discussed in section 3. Other modifications may also be required to tailor the general model to a particular data set. We recognize, for example, that our formulations of **Q** may be inappropriate. Bradford (1991) postulates a classification matrix **Q** with substantial under ageing of older fish. This leads to highly skewed probability distributions $q(a|b)$ for a given b. By contrast, the distributions in Fig. 2 are symmetric for true ages near the center of the age range.

We avoided the problem of small sample sizes at old ages by eliminating the oldest fish from our analysis. This may be impractical when old fish are important for biological interpretation. A common solution to this problem is to let age-class A denote all fish of that age and older. However, grouping older ages may necessitate a different representation for the final row

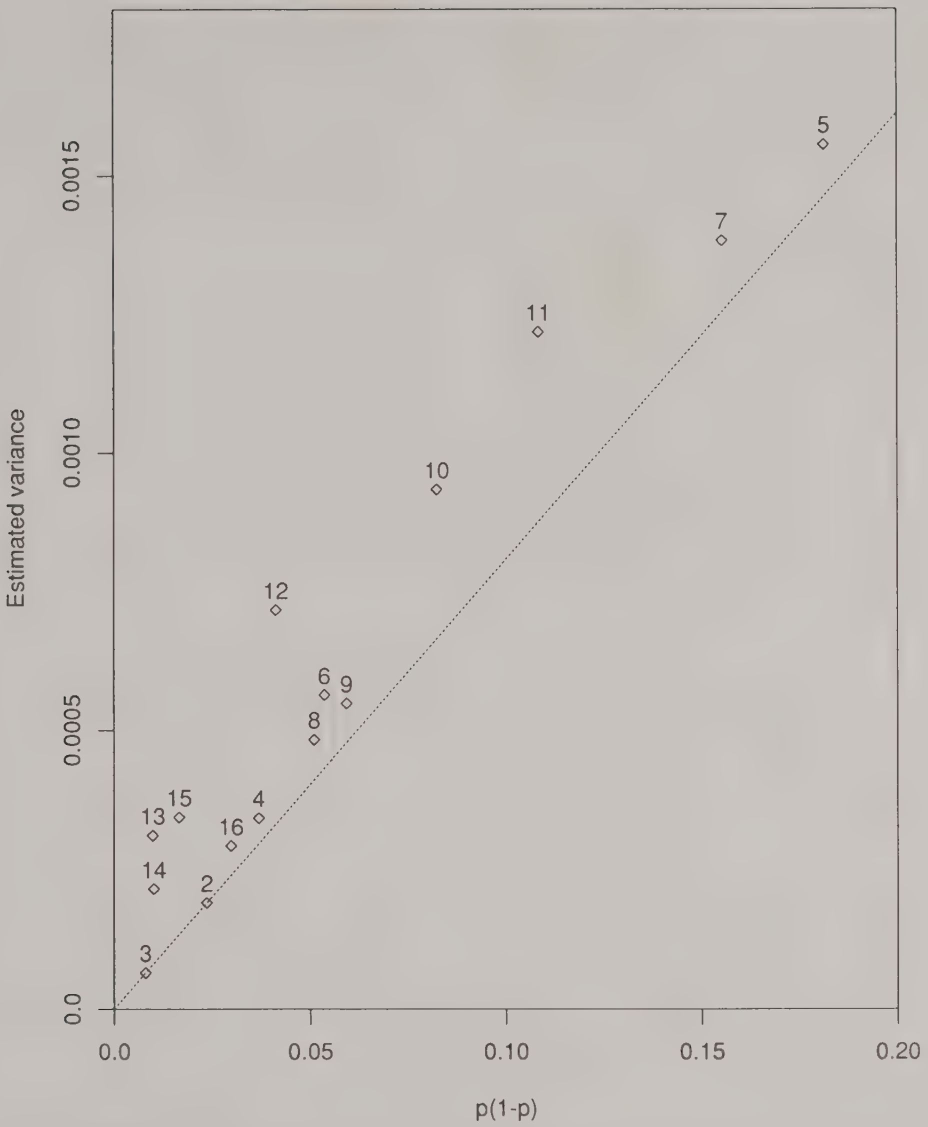

FIG. 5. Relationship between estimated variance $\hat{V}[\hat{p}_b]$ from (1.18) for the specified true age b and the product $\hat{p}_b(1 - \hat{p}_b)$. The dotted line has slope $1/I$, where $I = 124$, indicating the theoretical relationship for a multinomial distribution.

and column of **Q**. Grouping could also bias the estimates $\hat{p}_b$ in the vicinity of $b = A$, if the classification matrix estimated from one population is applied to another population with a different mix of older ages. In general, it is better to estimate $\hat{\mathbf{P}}$ from as many ages as possible. Ages can be grouped subsequently if required for other types of analyses. Models could also be designed using the concepts developed in (2.4), although technical implementation may be difficult.

Under most circumstances, it is not possible (or perhaps even desirable) to obtain precisely J readings of each age structure. However, the models can easily be generalized to account for unequal sample sizes. If J_i represents the number of readings for structure i, then from [4.5] the likelihood function to address question 3 is given by

$$(4.1)\qquad L(\mathbf{A}|\mathbf{P},\mathbf{Q}) = \prod_{i=1}^{I} \sum_{b=1}^{A} p_b \prod_{j=1}^{J_i} q(a_{ij}|b,\mathbf{\Phi}).$$

The likelihood [4.10] is a special case of (4.1), where $J_i = J$ for a subset of $I - K$ structures and $J_i = 1$ for the remaining K structures. Thus, the general likelihood function (4.1) can be used to address both questions 3 and 6.

In some situations, age increments from a specified date are known, but true ages are not. For example, distinguishing marks may naturally occur on ageing structures or may be induced with oxytetracycline injections (Thomas 1983; Beamish and McFarlane 1987; Casselman 1987). If fish are marked on a known date and later recovered, then two sets of readings on each age structure can be used to estimate a classification matrix in the context of question 2. For fish i and reader j, let a_{ij} and a'_{ij} denote estimated ages at recovery and mark formation, respectively. The likelihood for these data is given by

$$(4.2)\qquad L(\mathbf{A}, \mathbf{A}'|\mathbf{B},\mathbf{Q}) = \prod_{i=1}^{I} \prod_{j=1}^{J} q_j(a_{ij}|b_i,\mathbf{\Phi})\, q_j(a'_{ij}|b_i - t_i,\mathbf{\Phi})$$

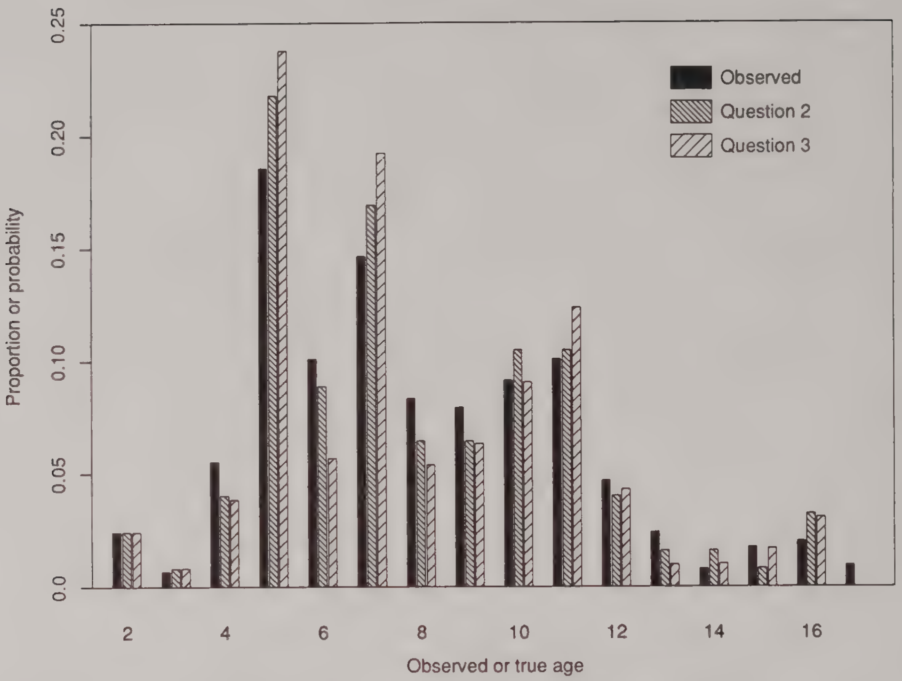

FIG. 6. Histograms relating ages a or b to observed proportions p_a^*, proportions p_b' estimated from $\hat{\mathbf{B}}$ in question 2 (Table 5, case 4), and estimated probabilities $\hat{p}_b$ in question 3 (Table 5, case 9).

TABLE 9. Estimates of reader effects ($r_1, \ldots, r_6$) in units of years for cases 11–14 in Table 5.

Case	$\hat{r}_1$	$\hat{r}_2$	$\hat{r}_3$	$\hat{r}_4$	$\hat{r}_5$	$\hat{r}_6$
11	0.120	0.015	−0.459	0.527	0.316	−0.519
12	0.130	0.014	−0.469	0.536	0.318	−0.529
13	0.078	0.006	−0.356	0.424	0.252	−0.405
14	0.082	0.005	−0.357	0.424	0.250	−0.404

where the matrix $\mathbf{A}'$ has elements a'_{ij}, t_i is the known time period between mark formation and recovery of fish i, and $q_j(a|b,\Phi)$ corresponds to a reader-dependent distribution as in (3.6). This representation assumes that readings are independent and that the values t_i are not revealed to readers.

Our models could also be modified to incorporate the case in which true ages are actually known for a sample of fish. Multiple readings from structures of known true age would then constitute a learning sample, and likelihood analysis would reduce to that familiar in mixture models (Fournier et al. 1984; Hoenig and Heisey 1987; Wood et al. 1987). In this case, a modal assumption would not be needed, since an adequate learning sample would provide the information necessary to estimate the entire classification matrix $\mathbf{Q}$. A model for $\mathbf{Q}$, such as (1.6)–(1.8), may still be desirable, however, to reduce the required number of parameters.

Our analysis does not specifically address the issue of experimental design. In section 2, we noted that additional readings of age structures (i.e. larger J) may provide increased precision in $\hat{\mathbf{Q}}$. Because ageing error tends to increase with age, additional readings may be particularly important for older ages. Thus, it may be appropriate to conduct a greater number of readings on age structures taken from fish presumed to be older. Similarly, larger random samples of structures (i.e. larger I) are required to achieve a specified precision in $\hat{\mathbf{P}}$ when ageing error is present, relative to a comparable data set with sampling error but no ageing error. Question 6 suggests one possible experimental strategy, in which multiple readings are performed on age structures from I fish, and age structures from an additional K fish are each examined once. These additional readings give increased precision in $\hat{\mathbf{P}}$. The likelihood (4.1) could be used to analyze all such experimental designs.

Among-reader variability is a well-known problem in age determination. For example, Kimura and Lyons (1991) document how the percentage agreement between two readers of a structure tends to decrease with increasing fish age. Percentage agreement also depends on the species. Averaging across ages, Kimura and Lyons (1991) report an agreement of 64% for walleye pollock, compared with 41, 44, and 79% agreement for Pacific ocean perch (*Sebastes alutus*), sablefish (*Anoplopoma fimbria*), and Pacific hake (*Merluccius productus*), respectively. Thus, for some species and readers, any single reading has a high probability of being wrong.

Ageing error corrections are applied infrequently in fisheries science, despite the known complications of ignoring this error. For example, Hollowed et al. (1987) and Myers and Drinkwater (1989) recognized the potentially confounding effects of ageing error in their attempts to relate year-class strength to environmental factors. Their analyses depended on previously published data, however, and they were unable to determine the influence of ageing error on their conclusions. Clearly, accurate ages are required to estimate year-to-year differences in fish survival and the dependency of survival on environmental factors. Our results (Fig. 6) reinforce the observation by several authors that ageing error acts to smooth differences in year-class strength, thus masking any such relationships.

Any method of age determination must be validated across all age-classes (Beamish and McFarlane 1987) before estimates of age and ageing error can be meaningful. Even with validation, however, there will generally be variability among readings of a given structure. In some cases, this variability is addressed by consultation, discussion, and perhaps gentle coercion in order to resolve disagreements in interpretation. It is then assumed that resolved ages represent true ages. Such an approach does not correct for ageing error; it merely standardizes an interpretation, and it may even lead to a false sense of precision. By contrast, our method uses variability among readings to assess statistical error. We exploit this knowledge to obtain corrected estimates of the true age distribution.

Our analysis provides an explicit means of dealing with the long-standing problem of measurement error in fish age determination. We believe that similar analyses should form a routine component of studies dependent on fish age data. In particular, multiple readings should be obtained for a subset of age structures and used to estimate the classification matrix **Q**. Single readings of additional structures can then help to improve precision in the estimate of **P**. We caution that, as ageing error increases, sample sizes must also be increased to obtain a specified level of precision.

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We wish to thank Dr. Daniel Kimura and the organizers and participants of the workshop on pollock ageing methodology, held from September 10 to 14, 1990, at the Sea Fisheries Institute, Gydnia, Poland. In particular, Dr. Jerzy Janusz provided one of us (R.J.B.) with the data used here. Thoughtful comments by an anonymous reviewer and Drs. Michael Bradford, John Hoenig, and Jake Rice helped improve the manuscript.

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Appendix

In this Appendix, we report technical details relevant to the estimates in Tables 5–8. Questions 2 and 3 are addressed by minimizing the inference functions (1.15) and (1.16), respectively. We applied the simplex search method (Nelder and Mead 1965; Press et al. 1986), as implemented by Mittertreiner and Schnute (1985). For question 2, the simplex method was used to obtain M of the parameter estimates, and the remaining I parameters were estimated by direct maximization. For question 3, all $M + A - 1$ parameters were obtained with the simplex method. In spite of the large number of parameters (up to 25), the simplex method performed adequately. For example, estimates for case 3 were achieved in 115 iterations from reasonable initial values; these required 3 min on a 486 33-mHz personal computer. Corresponding estimates for case 8 required approximately 4220 iterations (26 min). Individual iterations were more costly for question 2 due to the maximization (1.20)–(1.21). Total run time was much longer for question 3, however, because of the larger number of parameters involved.

The estimated asymptotic covariance matrix (1.18) was determined from numerical derivatives, where we follow the procedure described by Mittertreiner and Schnute (1985) to verify convergence. For these calculations, it is necessary to use the actual parameters p_b, rather than the surrogate parameters z_b in (1.22). Furthermore, the calculations must be performed twice, choosing a different age b_0 as the calculated parameter p_{b_0} in (1.23) for each run. Thus, $A - 1$ estimates $\widehat{\mathrm{SE}}(\hat{p}_b)$ can be obtained from the first run, but a second run is required to obtain the final estimate. Estimates $\widehat{\mathrm{SE}}(\hat{p}_b)$ are consistent between runs for the $A - 2$ unconstrained parameters p_b.

Analysis of Coded Wire Tag Returns from Commercial Catches

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Cormack, R. M., and J. R. Skalski. 1992. Analysis of coded wire tag returns from commercial catches. Can. J. Fish. Aquat. Sci. 49: 1816–1825.

Three alternative but equivalent approaches to the analysis of coded wire tag (CWT) data using log-linear models are presented. All three use iteratively weighted least squares to estimate treatment effects in hatchery releases under the assumption that the variance of a count is proportional to its expected value. The commonly made assumption of normal distributions with constant variance for recovery rates is inefficient. Analysis of tag recovery at the most disaggregated level (i.e. the level at which the sample fraction *f* is measured) is found necessary for valid inferences. Failure to include zero counts in analyses of recovery data is also shown to induce or mask interactions among CWT recoveries. Recoveries of CWT from coho salmon (*Oncorhynchus kisutch*) are used to illustrate the method of analysis. Coordinated CWT releases to facilitate mixing of stocks is recommended in the design of hatchery studies.

Nous présentons trois méthodes différentes mais équivalentes pour l'analyse des données sur les micromarques magnétisées codées à l'aide de modèles log-linéaires. Les trois méthodes font appel de façon itérative aux moindres carrés généralisés pour estimer les effets du traitement dans les lâchers de poissons d'élevage en prenant pour hypothèse que la variance d'un effectif est proportionnelle à sa valeur prévue. L'hypothèse couramment utilisée selon laquelle on obtiendrait des distributions normales avec une variance constante pour les taux de reprises n'est pas efficace. L'analyse des reprises d'étiquettes au degré le plus élevé de désagrégation (c.-à-d. niveau où on mesure la fraction *f* de l'échantillon) est nécessaire si l'on veut faire des inférences valides. Le fait de ne pas inclure les dénombrements zéro dans les analyses des données sur les reprises peut induire ou masquer des interactions parmi les reprises de poissons portant des micromarques. Nous nous servons pour illustrer la méthode d'analyse des reprises de micromarques implantées dans des saumons cohos (*Oncorhynchus kisutch*). Nous recommandons dans la conception des études sur les poissons d'élevage de coordonner les lâchers de poissons marqués pour faciliter le mélange des stocks.

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Much potential information about the contribution of hatchery and wild salmonids to Pacific coast fisheries is generated, at great expense, by intense programs of coded wire tagging (CWT). Millions of young fish have a CWT implanted in the nose cartilage before release. The tag identifies the fish to a specific treatment or release group from a specific hatchery or wild stock, with removal of the adipose fin as an external indicator of a tagged fish. Catches throughout the area are sampled, with widely varying levels of intensity, all fish in the samples being inspected for fin removal, with subsequent tag identification where possible. Thousands of tags are recovered annually, with numbers recovered identified as to location, time, gear type, etc., of capture. Data bases also include information on the sampling fraction f (or its reciprocal, the expansion factor e) of the stratum which was subjected to tag inspection. Despite all this effort and expense, there appears to be no consensus on the statistical methodology appropriate for extracting valid, useful information from such data. The monograph by Burnham et al. (1987) concentrates on studies with multiple recaptures. The extensive set of multinomial models developed by Brownie et al. (1985) for band-recovery studies concentrates on the separation of annual survival rates from recovery data within a program of multiple releases into a single population over several years. Their extension by Schwarz et al. (1988) to incorporate postrelease stratification, often at time of recovery, brings them closer to the special requirements of data from commercial fisheries, but still with a different emphasis and no formulation of the problem of subsampling the catch. Green and Macdonald (1987) advocated the use of log-linear models fitted using the computer package GLIM (Baker and Nelder 1978), analysing the observed counts as independent Poisson variables and summarising the biological structure in models for the expectation of these variables. The aim of this paper is to give strong support to the approach of Green and Macdonald (1987) and to discuss some aspects of such analyses, in particular the effects of aggregating or compositing different recovery units. Computer packages other than GLIM have programs for log-linear models but with less immediacy in their interactive nature.

The fundamental unit of recapture is a collection of fish caught in a specific type of fishery within a geographic region and over a specified duration of time that was inspected for tagged fish. Although the collection may extend over a long period of time and a wide spatial area, it is this fundamental sampling unit that provides a datum for tag analysis. Denote by n_{ij} the number of fish with code i, from an initial release of R_i fish with that code, that are observed in the inspected sample from catch unit j. Then the expected value of n_{ij} is

TABLE 1. Notation used in the analysis of CWT data.

Type	Symbol	Definition
Indices	i	Label for different tag release groups
	j	Label for different catch units
	$+$	Replaces a label when it is summed over
Fixed Number	R_i	Number released bearing tag code i
	f_j	Proportion of catch j inspected for tags
	$e_j = 1/f_j$	Expansion factor for catch j
Observations	n_{ij}	Number of fish with tag code i found in the inspected sample from catch j
	$E_{ij} = n_{ij}\, e_j$	Expanded number of tags code i in catch j
	$y_{ij} = n_{ij}\, e_j/R_i$	Recovery rates of tag code i in catch j
Parameters	θ_{ij}	Probability that a fish with tag code i is caught in catch j
	$\mu_{ij} = R_i f_j \theta_{ij}$	$E\,(n_{ij})$: number of fish with tag code i expected to be found in the sample inspected from catch j
	${\sigma_{ij}}^2$	Var (n_{ij}): variance of n_{ij}
	$\phi = {\sigma_{ij}}^2/\mu_{ij}$	Variance-to-mean ratio for n_{ij}, when this is assumed constant

$$E(n_{ij}) = R_i \theta_{ij} f_j$$

where θ_{ij} is the probability that a fish with code i contributes to catch j and f_j is the fraction of catch j that is sampled. From the data (n_{ij}, R_i, f_j), a statistical model for the n_{ij} is selected in order to find a parsimonious description of the θ_{ij} in terms of a structure on the release suffix i (different hatcheries, experimental treatments, etc.) and the recovery suffix j (type, location, and timing of fishery, etc.) and to give point estimates and confidence intervals for the θ_{ij} and appropriate combinations of them. Although combinations of the θ_{ij} may be of particular interest, we recommend modelling and analysing the data (n_{ij}) at the fundamental, most disaggregated level at which the sampling fractions f_j are defined. The consequences of trying to analyse aggregated data are considered later. For convenience, all notation is summarised in Table 1.

Statistical Model

Variance Structure

Since R_i is typically very large and θ_{ij} small, a natural first choice of model would be to assume independent Poisson distributions for the n_{ij}. This model explicitly assumes that different sampling strata do not interact and implicitly assumes that individual fish behave independently with respect to the overall process from release to capture and sampling. Overall validity of the Poisson assumption can be tested, and possible interactions between specific fisheries (e.g. between a rivermouth and an upstream fishery) can be examined in terms of the correlation between the residuals from the model. Shoaling behavior of fish and the fact that fishermen respond to it are just two of many reasons to suggest that some clustered distribution would be expected to provide a better fit than a Poisson model. The mechanisms of sampling and subsampling of the catch are also likely sources of additional variability. The overall result, commonly experienced, is that the variance of true replicate counts tends to be greater than their mean.

For a Poisson distribution, the variance equals the mean. The variance to mean ratio, $s^2/\bar{x}$, among k replicate counts is $1/(k - 1)$ times the index of dispersion, widely used in ecology as a test of nonrandomness (i.e. $s^2\,(k - 1)/\bar{x} \sim \chi^2_{k-1}$; Ripley 1981, p. 104). How should data be analysed when there is an a priori expectation of overdispersion and evidence from the data that this expectation has been fulfilled? Three approaches can be distinguished: (1) ignore the variance structure, (2) develop a detailed model of the overdispersion, and (3) make inferences about the parameters θ_{ij} using a robust method which allows for overdispersion without modelling it in detail. We will discuss each of these approaches below.

The first approach comes in three forms. In the first of these (Table 2, Model 1), the recovery rates $y_{ij} = n_{ij}\, e_j\,/R_i$ are regarded as normally distributed variables with constant variance. This neglects both the likely relationship between variance and mean and the necessary dependence of the variance on both the number released and the expansion factors. It also ignores the fact that a normal distribution with constant variance is virtually impossible for any nonnegative variable which can be very small. A variant of this (Table 2, Model 2) assumes $\log\,(y_{ij})$ to be normally distributed with constant variance. This overcorrects the dependence of Var (n_{ij}) on number released and on the expansion factors, and in implying that the variance of y_{ij} is roughly proportional to the square of its mean is likely to give too little weight to large observations. Model 2 also has the difficulty of requiring an arbitrary modification when the return rate is zero. The third form ignores the likely presence of overdispersion, assuming a Poisson distribution with variance equal to mean. Although in our opinion much superior to the normality assumptions above, this assumption leads, in the presence of any overdispersion, to understatement of the variance of estimated effects and the overstatement of the significance of possible explanatory factors.

If the primary interest lies in the causes of the variability in the number of tags recaptured within a stratum, then the second approach is necessary. This would require special experimentation and be very difficult. Assuming that the observations follow, say, a negative binomial distribution — often used as a description because it can be derived from a number of ecological processes, although not if more than one is operating — is not developing a detailed model. Making an inference which depends specifically on the distributional form of the negative binomial is dangerous because the data seldom provide adequate evidence for or against the negative binomial rather than any other clustered distribution. The property which is important and about which the data set does provide some evidence is that its variance $\sigma^2 = \mu + \mu^2/k$ when its mean is μ.

Following Green and Macdonald (1987), we recommend the third approach. We make no assumption about the probability distribution of the observations n_{ij}, but do assume that their variances ${\sigma_{ij}}^2$ are related in a specified way to their means μ_{ij}. This relation can include an unknown parameter to be estimated from the data. The negative binomial form $(\mu_{ij} + {\mu_{ij}}^2/k)$ would be one possibility, but there are theoretical and computational advantages in including the extra parameter as a scale factor, taking ${\sigma_{ij}}^2 = \phi\mu_{ij}$ where $\phi > 1$ represents overdispersion. This forms Model 3A (Table 2) called a scaled Poisson by Green and Macdonald (1987) although, taken literally, a Poisson variable multiplied by a scale factor in general takes values that are not integers. Although we prefer to consider this as a model for n_{ij}, the number of tags actually observed, the same form of model applies to the recovery rate y_{ij} (Model 3C) or, as used

TABLE 2. Possible statistical models used in the analysis of CWT data.

Model	Dependent variable	Mean	Variance	Error distribution	Fitting constants	
					Weight	Offset
1	y_{ij}	θ_{ij}	Constant	Normal	1	None
2	$\log (y_{ij})$	$\alpha_i + \beta_j + \gamma_{ij}$	Constant	Normal	1	None
3A	n_{ij}	$\mu_{ij} = R_i \theta_{ij} f_j$	$\phi\mu_{ij}$	Scaled Poisson	1	$\log (R_i f_j)$
3B	E_{ij}	$R_i\theta_{ij}$	$\phi\theta_{ij} e_j$	Scaled Poisson	f_j	$\log (R_i)$
3C	y_{ij}	θ_{ij}	$\phi\theta_{ij} e_j / R_i$	Scaled Poisson	$R_i f_j$	None

by Green and Macdonald (1987), to the expanded catch E_{ij} (Model 3B), since these are known multiplies of n_{ij}. The multipliers have to be incorporated as weights in any analysis.

The choice between the two forms of variance function, negative binomial and scaled Poisson, is not as important as maybe first thought. Over the range of means encountered at the disaggregated level, they may not be readily distinguishable. Furthermore, there exists evidence that "even relatively substantial errors in the assumed functional form of Var(Y) generally have only a small effect on the conclusions" (McCullagh and Nelder 1989, p. 199). It should be noted also that the structure $\sigma_{ij}^2 = \phi\mu_{ij}$ does not greatly restrict the probability distribution of the counts: any family of discrete distributions indexed by two or more parameters includes members which take this structure.

Formulated in this way, the variance of n_{ij} incorporates the variability in survival of young fish between release and capture in the fishery, in sampling the catch, and in extraction and recording of the tag. These variances will be greater than those presented by Clark and Bernard (1987) which incorporated only the subsampling of the catch. Their variances are appropriate to estimation of past catch in a given time period. If the complete catch were counted, then the size of that catch and its component parts would be known exactly. However, one would not know exactly the relative contributions to be expected from different hatcheries in another year, nor would one quote with zero variance the differential effects of two treatments (e.g. time of release) on the fishery return.

Method of Fitting

For data at the fundamental level of disaggregation, the time–area fishery stratum over which f_j was originally measured, the observed number n_{ij} of tag recaptures has expected value (Table 2, Model 3A)

$$E(n_{ij}) = \mu_{ij} = R_i\theta_{ij} f_j .$$

This is a log-linear model

$$\log (\mu_{ij}) = \log (R_i f_j) + \log (\theta_{ij})$$

with associated variance Var $(n_{ij}) = \phi\mu_{ij}$ where ϕ is an unknown constant of proportionality. The $\log(\theta_{ij})$ can be further partitioned by an additive factorial model representing the structure of the study, for example, release treatments or regions and times of recapture.

Most statistical packages contain options for fitting log-linear models to counts n_k, assumed Poisson, with means μ_k given by

$$\log (\mu_k) = \sum_l \beta_l x_{kl} .$$

In our case, one of the x-variables is $\log(R_i f_j)$ whose regression coefficient is forced to be 1. Such a variable is said to be offset. A referee has pointed out that if $\log(R_i f_j)$ is written as $\log(R_i) + \log(f_j)$ and only $\log(f_j)$ is offset, the regression coefficient of $\log(R_i)$ would reflect density-dependent mortality of the release groups (Peterman 1982). The other factors are indicator variables, zero or one, for the different categories of release and recapture, but covariables such as effort can be included provided they can be modelled within the log-linear form. Analysis of E_{ij} or y_{ij} requires different offsets, given in Table 2, and also the use of weights to adjust the variances, as discussed by Green and Macdonald (1987). The algorithm in GLIM converges more slowly for Model 3C. Maximum likelihood estimates $\hat{\beta}_l$ are obtained, together with their asymptotic standard errors. The overall fit of any model is assessed by comparison of observations n_k with their fitted values $\hat{\mu}_k$ by means of the deviance

$$2 \sum_k n_k \log \left(\frac{n_k}{\hat{\mu}_k} \right) - 2 \sum_k (n_k - \hat{\mu}_k).$$

If the model includes a constant term, the second term in this expression is zero. See, for example, Green and Macdonald (1987) or McCullagh and Nelder (1989, Section 2.3).

Different nested models for $\log (\theta_{ij})$ can be compared, in a way exactly parallel to the analysis of variance of a factorial experiment, by examination of differences between the deviances from different models. Estimates and asymptotic variances and covariances of $\log (\theta_{ij})$ under the selected model yield normal confidence intervals on this scale. To obtain confidence intervals for a function of a linear combination of $\log (\theta_{ij})$, for example, the relative contribution of two treatments $\theta_{1j} / \theta_{2j}$ to a fishery, limits for the function on the log scale are converted by exponentiation to limits for the required function. Confidence limits for sums or differences of θ_{ij} can be approximated by the assumption of a normal distribution for $\hat{\theta}_{ij}$, with variance obtained by the usual Taylor series expansion of the appropriate function of the estimators of $\log \theta_{ij}$ (Seber 1982, p. 7–9).

With the Poisson distribution, the above log-linear model belongs to the class of generalized linear models (GLMs) introduced by Nelder and Wedderburn (1972). They showed that the maximum likelihood estimates could be obtained by a weighted least squares procedure, where the weights are determined iteratively by the variance of each observation, a procedure which makes no use of the specific probability distribution.

When the Poisson distribution is not assumed, and the additional unknown parameter ϕ is introduced as the constant variance-to-mean ratio, the same method of analysis can still be used. The theoretical difference is that the justification is now in terms of weighted least squares and quasi-likelihood (McCullagh and Nelder 1989, Chapter 9). The only practical differences are that: (1) since ϕ is no longer known to be 1, it has to be estimated by $\hat{\phi}$, the Pearson goodness-of-fit χ^2 from the most complicated model considered, divided by its degrees of freedom ($\hat{\phi} = \chi^2/\text{df}$): if this is less than unity, however, we take $\hat{\phi} = 1$, since underdispersion is a priori unlikely, (2) standard errors of estimates must be multiplied by $\hat{\phi}^{1/2}$, and (3) in model selection, differences between deviances should be divided by $\hat{\phi}$ before comparison with the appropriate χ^2 distribution (Breslow 1990). These recommendations for practical data analysis are discussed further in the final section of the paper.

Potential difficulty in the analysis of CWT data can occur if the number n_{ij} is zero. Zero observations are real data and should be included in the analysis, particularly when comparisons are being made between treatment groups, some of which make a nonzero, some a zero, contribution to a particular recovery stratum. Even if zeroes are not recorded in a data base, they may be implied by other records and should not be ignored. For example, a data base may record one fish from hatchery H_3 recovered in area A_2 during period W_3. The absence of any mention of a fish from hatchery H_2 being recovered in A_2 during period W_3 implies a zero record for that combination. However, before drawing the corresponding conclusion from the absence of any record from H_3 in A_1 during W_3, we must be sure that samples were taken in A_1 during W_3.

The situation in which no tagged fish at all are recorded in a particular stratum requires further consideration. If the structure of the model assumed for θ_{ij} contains a separate parameter for that stratum, then the fitted values for all observations in that stratum will also be zero. This stratum thus makes zero contibution to the χ^2 goodness-of-fit and would reduce the estimate of ϕ if the df were not correspondingly reduced, giving a false precision to inferences about other strata. The stratum as a whole should be removed from the analysis. If the model for θ_{ij} results in small nonzero fitted values, a satisfactory resolution is less straightforward. We suggest that the data be reanalysed with such sets of zeroes omitted and any major difference in the outcomes of the two analyses regarded as a need for cautious interpretation.

Aggregation

Methodology of previous sections concentrates on inferences about the contribution of a particular treatment release to a particular recovery stratum. Notation was kept simple by allocating a single index j to all recovery strata, However, information is often wanted on aggregations of these fundamental units, aggregations over any combination of time, area, gear type, or complete fishery. Notation has to be extended to allow at least the minimum structure on strata. To make it specific, we denote by n_{ijk} the number of tags from release i recaptured in area j during week k while noting this, or a more general notation, can be applied to any known structure on the recoveries or the releases. Interest lies in area totals. The observed numbers n_{ijk} and the expanded numbers $E_{ijk} = n_{ijk}e_{jk}$ are added up to obtain area totals: $n_{ij+} = \sum_k n_{ijk}$ and $E_{ij+} = \sum_k E_{ijk}$. Indeed, these totals are sometimes all that are reported in derived analyses. The individual sampling fractions have been discarded and what is reported is an aggregated expansion factor $e_{ij} = E_{ij+}/n_{ij+}$ which is itself now a random variable. What analyses are possible using only the aggregated values n_{ij+}, E_{ij+}, and hence, e_{ij}?

Corresponding to Model 3A, n_{ij+}, aggregated over some fisheries for a single release, is distributed with mean $\mu_{ij+} = R_i \sum_k (\theta_{ijk}/e_{jk})$, a function which depends on the discarded individual expansion factors e_{jk} and consequently does not yield information about θ_{ijk} or $\theta_{ij+} = \sum_k \theta_{ijk}$. On the other hand, E_{ij+} has expectation $R_i\theta_{ij+}$, and $y_{ij+} = E_{ij+}/R_i$ has expectation θ_{ij+}, which is the probability of a fish from code i contributing to the fishery in area j. Thus, with aggregated data, Models 3B and 3C describe variables whose expected values are of interest whereas Model 3A does not. However, an analysis which is fully valid requires knowledge of how the variance of each variable is related to its mean under the model. Now

$$\text{(1)} \qquad \begin{aligned} \text{Var}\,(y_{ij+}) &= \frac{\phi \sum_k \theta_{ijk}e_{jk}}{R_i} \\ \text{Var}\,(E_{ij+}) &= \phi \sum_k R_i\theta_{ijk}e_{jk} \end{aligned}$$

so that in both cases, the variance depends on the θ_{ijk}, which cannot be estimated from the aggregated data, and on the e_{jk}, which have been discarded. Thus, no analysis can be based on an exact model for the mean and variance of aggregated data.

Can an approximation to the variance be found in terms of the known e_{ij} and the parameter of interest θ_{ij+}? Formula (1) can be rewritten as follows:

$$\begin{aligned} \text{Var}\,(y_{ij+}) &= \left(\frac{\phi}{R_i}\right) \sum_k \theta_{ijk}\,[e_{ij} + (e_{jk} - e_{ij})] \\ &= \frac{\phi e_{ij}\theta_{ij+}}{R_i} + \left(\frac{\phi}{R_i}\right) \sum_k \theta_{ijk}\,(e_{jk} - e_{ij}) \end{aligned}$$

with a corresponding form for Var (E_{ij+}). If the second term is negligible, these are Models 3C and 3B of Table 2, at the aggregated level.

If all the expansion factors within an area are the same for different weeks, the second term vanishes and analysis of Model 3 at the aggregated level is valid. Otherwise, since e_{ij} is a weighted average of the e_{jk}, the second term depends on the covariance or correlation between θ_{ijk} and e_{jk}. Observations on these have been discarded. Since θ_{ijk} is a parameter of the process linking release to capture in a fishery, and e_{jk} is a parameter of the sampling process after capture, this correlation would be expected to be small on average. However, for a particular treatment group i, it can be substantial, of either sign. Consider, for example, the effect of severe undersampling in one week in one area, thus generating a high e_{jk} $(= 1/f_{jk})$. Then a treatment group that is expected to make a higher than average contribution to that stratum will have a variance higher than that indicated by the model, and conversely for a group making a low contribution to that stratum. No information on this situation is available from the aggregated data alone, and this phenomenon may add greatly to the noise already distorting empirical attempts to model variance–mean relationships of aggregated data. The effects will be demonstrated in the next section.

By contrast, aggregation over treatment groups creates no problems for analysis. Interest lies in $\theta_{Ij} = \sum_{i\in I} R_i\theta_{ij} \Big/ \sum_{i\in I} R_i$, the average contribution of fish in release I to the jth recovery stratum weighted by the number of fish of each type in the aggregated release I. Under the model, the total of the observed numbers $n_{ij} = \sum_{i\in I} n_{Ij}$ has expected value

TABLE 3. CWT releases of coho reported in Clark and Bernard (1987) and recoveries (n_{ij}) by area and week.

			Treatment:	1		2		3	
Area	Week	Expansion factors	No. released:	10 020	10 454	10 349	10 159	10 774	10 630
1	1	65.08		0	2	3	1	1	2
	2	5.98		2	3	7	4	3	1
	3	5.82		7	7	15	7	7	6
	4	4.37		39	27	38	45	9	15
	5	4.64		6	13	20	8	4	3
2	1	5.13		0	0	1	0	2	0
	2	3.76		4	3	1	3	5	1
	3	3.72		3	8	3	3	0	1
	4	3.99		4	4	5	6	2	3
	5	4.64		6	6	9	11	4	4
3	1	6.35		1	0	0	0	1	0
	2	2.84		0	1	1	1	0	0
	3	3.63		0	1	0	0	0	1
	4	4.22		1	1	5	5	1	1
	5	6.30		0	0	0	1	0	0
4	1	2.76		1	1	0	0	0	0
	2	3.38		0	1	0	2	1	1
	3	2.15		2	0	1	5	1	4
	4	2.73		5	4	5	16	3	7
	5	5.72		4	3	9	7	4	3

$$\mu_{Ij} = f_j \sum_{i \in I} R_i \theta_{ij} = f_j R_I \theta_{Ij}$$

and variance $\phi\mu_j$ where $R_I = \sum_{i \in I} R_i$. This is identical in form to Model 3A. Similarly, E_{Ij} and y_{Ij} have the correct structures for Models 3B and 3C.

The problem arises from the aggregation of unequal sampling fractions. Although aggregation of samples might be regarded as just another level of clustering of fish, the sampling of the catch introduces a different and difficult element into the analysis. We can gain some understanding of the variance structure only at the disaggregated level.

Example

Clark and Bernard (1987) quoted data on recaptures from five releases, two of chinook salmon (*Oncorhynchus tshawytscha*) and three of coho salmon (*O. kisutch*), from Whitman Lake Hatchery. Ten tag codes were used, two tag codes within each release group, because of insufficient supplies of tags of any one code. Recoveries from the southeast Alaska fisheries in one year are listed at a highly disaggregated level, the strata being defined by type of fishery (troll, seine, gill net, or fishtrap), by region, and by period of recovery. Since there are no recovery regions in common between the troll and other fisheries, and a high degree of further nonorthogonality in regions and periods between the other fisheries, it would be difficult to interpret a single all-embracing analysis. To demonstrate the methodology and its differences from other approaches, we analyse here data on the recoveries from the troll fishery of coho for which recoveries are recorded in four regions (A = 1, . . ., 4) and five weekly sampling periods (W = 1, . . :, 5), regarding the three separate releases as three hatchery treatments (T = 1, . . ., 3), each having two tag codes. The first of these treatments differed from the others in being fish of a different stock released in a different place. The difference between second and third treatments is not defined by the authors. Each recovery stratum is indexed by region and week.

The coho data are given in Table 3 in a form that differs in certain respects from the originals in Clark and Bernard (1987, appendix E, tables 4, 6, and 8). The expansion factor for each recovery stratum has been deduced from the estimated contributions of each release to each fishery quoted by Clark and Bernard (1987). When no fish from either tag code of a treatment are observed in a stratum, such double zeroes are not listed explicitly in Clark and Bernard (1987). In Table 3, such zeroes are included with expansion factors inferred from the nonzero observations in that stratum from some other release. If other strata were fished and sampled but no tags were observed from any release, no information on such strata is available from Clark and Bernard (1987). The range of expansion factors, from 2 to 65 in the coho study, is not atypical. The roughly equal numbers of fish released in each group is atypical but not crucial for the analysis.

Recommended Analysis

Five different models for θ_{TAW}, the probability that a tagged fish from treatment group T is caught in region A during week W, are listed in decreasing order of complexity in Table 4. Measures of lack-of-fit of these models to the data are also listed: deviances and standard Pearson χ^2. Score tests, easily implemented in GLIM (Pregibon 1982), are an alternative to the tests based on deviance differences: they give very similar results. Model 1, the most general, allows a different value of θ for each treatment–region–week combination by allowing all interactions among the three factors (indicated by notation T*A*W). The fact that χ^2 is a little less than the residual df indicates that a Poisson distribution may be acceptable, i.e. with proportionality factor ϕ = 1. This confirms that the observed variance is the result of process variability (random survival, catch, and sampling) alone. Fish seem to behave

TABLE 4. Goodness-of-fit of models and tests of model differences for the six CWT releases of coho reported in Clark and Bernard (1987). Factors T, A, and W denote treatments, regions, and weeks, respectively; model $T + A$ includes main effects only, while $T*A$ also includes their interaction.

	Goodness-of-fit			Differences		
Model	Deviance χ^2	Pearson χ^2	df	Deviance	Pearson	df
Full: $T*A*W$	62.84	54.74	60			
θ_{TAW} all different				30.76	34.61	24
All 2-factor interactions						
$T*A + T*W + A*W$	93.60	89.35	84			
$\theta_{TAW} = \delta_{TA}\beta_{AW}\gamma_{TW}$				9.15	15.75	8
$T*A + A*W$	102.75	105.1	92			
$\theta_{TAW} = \delta_{TA}\beta_{AW}$				9.72	10.20	6
$T + A*W$	112.47	115.3	98			
$\theta_{TAW} = \delta_T \beta_{AW}$				94.62	124.70	12
$T + A + W$	207.09	240.0	110			
$\theta_{TAW} = \delta_T \beta_A\gamma_W$						

homogeneously and independently as they move through the process from their hatchery towards the fishery. Further models now explore differences and interactions between treatments, regions, and weeks of fishing. Model 2 sets the three-factor interaction to zero, and other interactions are removed in successive lines of Table 4 until the no-interaction Model 5 (i.e. $\theta_{TAW} = \delta_T \beta_A\gamma_W$) is reached.

At each stage from Model 2 onwards, other models were considered and the route chosen which minimised the increase in mean deviance (deviance/df). Thus, for example, Model 2 includes three two-factor interactions — treatment by region ($T*A$), treatment by week ($T*W$), region by week ($R*W$) — each of which was removed from the model in turn. Model 3 was chosen next because the interaction between T and W was least significant. The procedure is akin to backward selection of variables in multiple regression, although with three factors, all 19 hierarchical models can easily be fitted.

Model 5 is clearly unacceptable, since the difference in deviance between it and Model 4, a value of 94.6, is unacceptable as a value from a χ^2 distribution with 12 df ($P(\chi_{12}{}^2 > 94.6) \ll 0.001$) as is the residual χ^2 of 240.0 from a χ^2 distribution with 110 df ($P(\chi_{110}{}^2 > 240.0) \ll 0.001$). For all other models, both the overall χ^2 and the differences between deviances from successive models take values which are acceptable when assessed against the χ^2 distributions. We select as the most parsimonious acceptable Model 4: $\theta_{TAW} = \delta_T\beta_{AW}$ (where δ_T is the "effect" of release T and β_{AW} the "effect" of the fishery in region A, week W). For Model 4, the value of the Pearson χ^2 is acceptable ($P(\chi_{98}{}^2 > 115.3) = 0.11$) as is the cumulative difference in deviance between Model 1 and selected Model 4 ($P(\chi_{38}{}^2 > 49.6) = 0.10$).

Selection of an acceptable model on the basis of the asymptotic distribution of the deviance D or χ^2 may be questioned because of the small expected counts in several cells. Despite considerable work on the problems of sparse data, "general rules are difficult to prescribe for unsymmetrical null hypotheses" (Koehler and Larntz 1980). "It is good statistical practice not to rely on either D or χ^2 as an absolute measure of goodness-of-fit in these circumstances. it is much better to look for specific deviations from the model of a type that is easily understood scientifically" (McCullagh and Nelder 1989, p. 122).

Although Model 4 is acceptable with $\phi = 1$, its estimate is $\hat{\phi} = 115.3/98$ or 1.177. Since we argue that scientifically some clustering of fish is to be expected, we advocate using $\hat{\phi}$ in constructing confidence intervals. Failure to reject a null hypothesis is not proof of its truth. Model 5 is still overwhelmingly rejected compared with Model 4.

A desirable supplement to any analysis is to examine the residuals of observations from their fitted values under the model. Since our observations do not have constant variance and are not symmetrically distributed, a transformation is needed to give readily interpretable residuals. Pearson residuals:

$$r = \frac{(n - \hat{\mu})}{\hat{\mu}^{\frac{1}{2}}}$$

or Anscombe (1953) residuals:

$$r = 1.5\,\frac{\left[n^{\frac{2}{3}} - \hat{\mu}^{\frac{2}{3}}\right]}{\hat{\mu}^{\frac{1}{6}}},$$

the latter specifically designed for Poisson distributions, are very similar unless $\hat{\mu}$ is very small. The normal plot of r (Fig. 1a) shows no disturbing features.

A further safeguard would be to check the fit of the selected model by Monte Carlo simulation of Poisson observations with cell means given by the fitted values from the real observations and subsequent fitting of the same model to the simulated data sets. If fewer than 5% of the simulations result in a goodness-of-fit value greater than that calculated from the real data, the model would be rejected at the 5% level. In 200 simulations of Model 4, 25 or 12.5% had a deviance larger than the observed 112.5. Although not significant, this again suggests that some extra-Poisson variation may exist or that some other interaction term may be important. The mean and variance of the simulated deviances were 97.1 and 168 compared with the values of 98 and 196 for the assumed χ^2 distribution with 98 df.

Even if $\phi > 1$, the selected model indicates that, although the relative contributions of the different regions to the overall fishery change from week to week, the contributions of δ_T of each treatment code to every catch remain in the same ratio. In

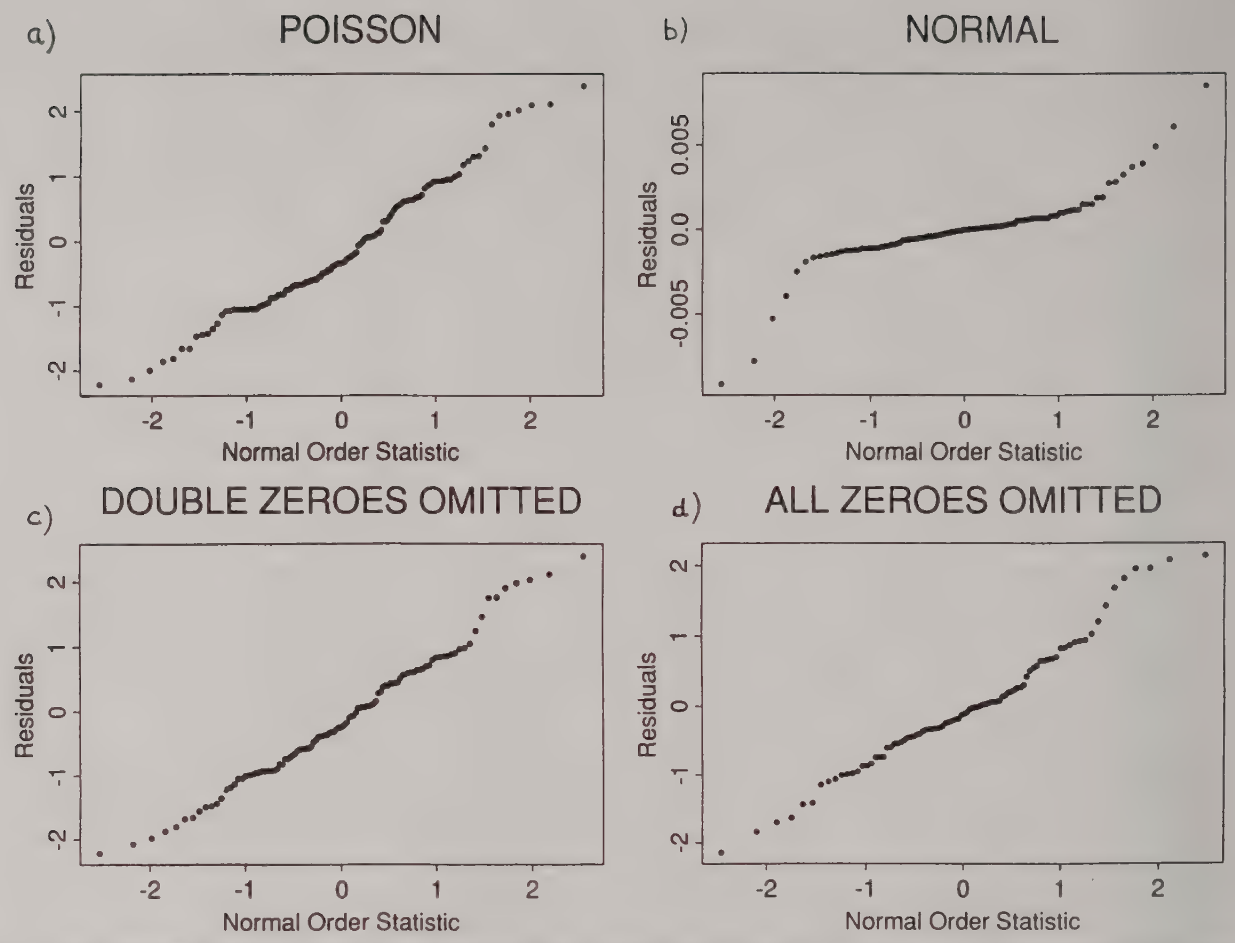

FIG. 1. Plots on normal probability paper of Anscombe-transformed residuals from the model $\theta_{TAW} = \delta_T \beta_{AW}$ to Clark and Bernard (1987) coho data. If the assumptions of the analysis are satisfied, the plot should be a straight line. (a) Residuals from the complete data fitted by the recommended model with observed recoveries assumed Poisson distributed; (b) residuals from the complete data fitted assuming observed recovery rates normally distributed with constant variance; (c) as in Fig. 1a but all categories omitted with no recoveries from any tag code; (d) as in Fig. 1a but all categories with no recoveries omitted.

other words, there seems to be reasonably uniform mixing of the various treatment groups over both space and time. The estimates of ($\log \delta_2 - \log \delta_1$) and of ($\log \delta_3 - \log \delta_1$) with their standard errors, incorporating $\hat{\phi}^{1/2}$, are respectively 0.376 ± 0.100 and −0.565 ± 0.126. Thus, on exponentiating, estimates are that for every 100 fish contributed to any recovery class from fish released in the first treatment, the same number of fish released by the second treatment will contribute 146 fish to that recovery class (95% confidence limits 118 to 180), while the same number of fish released by the third treatment will contribute 57 fish to that class (95% confidence limits 44 to 74).

Since the selected model ($\theta_{TAW} = \delta_T \beta_{AW}$) represents complete mixing of treatment groups of fisheries, an alternative simple comparison between treatment effects can be based on the observed counts, ignoring expansion factors. Merely dividing observed recoveries by numbers released on each treatment group gives estimated recovery rates respectively of 0.0083, 0.0121, and 0.0047 with $\log(\delta_2/\delta_1)$ estimated as 0.376 and $\log(\delta_3/\delta_1)$ as −0.565. These are identical to the values in Table 4 obtained by the log-linear analysis of the disaggregated data with variance proportional to mean and different from any analysis involving aggregation of expansion factors. The theoretical binomial standard errors of these comparisons are 0.099 and 0.125, respectively, the first being slightly less than the standard error from the log-linear analysis. Sample standard errors based on the difference between recovery rates from the duplicate codes are less than half the theoretical values, but being based on 1 df are highly unreliable and give wide confidence limits.

It should be noted that overdispersion and lack-of-fit cannot be distinguished from any overall measure. Perhaps Model 5 is acceptable with a large value of ϕ. The evidence against this lies in the pattern of residuals from Model 5 (Table 5). For example, Area 1 is high in Weeks 1, 2, and 3 and low in Week 5; Area 4 is low in Week 1 and high in Week 5.

Alternative Analyses

Other analyses which have been suggested in the literature were discussed earlier. What results do they give for this example?

Omitting zero catches

If the analysis is repeated omitting the region–week classes with no recoveries from any code, the results are very similar.

TABLE 5. Anscombe residuals of n_{ij} from Model 5: $T + A + W$ for the Clark and Bernard (1987) coho data.

		Treatment 1		Treatment 2		Treatment 3	
Area	Week	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	1	−0.8	2.1	2.6	0.8	1.4	2.6
	2	−1.0	−0.5	0.5	−0.7	0.4	−1.0
	3	0.2	0.1	1.5	−0.9	1.4	1.0
	4	2.7	0.3	0.2	1.4	−1.7	0.1
	5	−2.4	−0.4	−0.1	−3.1	−1.7	−2.1
2	1	−1.5	−1.5	−0.4	−1.8	1.5	−1.1
	2	1.5	0.9	−1.1	0.3	2.9	0.0
	3	0.1	2.5	−0.6	−0.6	−2.0	−0.6
	4	−1.3	−1.4	−2.0	−1.6	−1.3	−0.7
	5	1.1	1.0	1.3	2.1	1.0	1.1
3	1	1.4	−0.6	−0.8	−0.8	1.7	−0.5
	2	−1.1	0.6	0.3	0.3	−0.8	−0.8
	3	−1.2	0.4	−1.5	−1.5	−0.9	0.8
	4	0.5	−0.5	1.5	1.5	0.0	0.1
	5	−1.2	−1.2	−1.5	0.1	−0.9	−0.9
4	1	−0.2	−0.2	−2.0	−2.0	−1.3	−1.3
	2	−1.6	−0.2	−2.0	0.2	0.3	0.3
	3	−0.7	−2.7	−2.1	0.2	−0.7	1.3
	4	−0.8	−1.3	−1.9	1.7	−0.6	1.3
	5	1.3	0.6	2.8	2.0	2.0	1.4

TABLE 6. Test statistics for model $\theta_{TAW} = \delta_T \beta_{AW}$ for the Clark and Bernard (1987) coho data.

	Test of interaction			Test of residual		
Data form	df	Change in deviance	Change in χ^2	df	Deviance	χ^2
Diaggregated	38	49.6	60.6	60	62.8	54.7
Disaggregated (all zero counts ignored)	30	29.2	34.3	39	39.3	37.8
Aggregated over weeks	6	5.1	5.2	12	16.5	16.6
Aggregated over regions	8	18.2	15.0	15	24.9	31.2
Aggregated over both		None possible		3	6.6	6.7

The residual χ^2 from the full model $T*A*W$ remans approximately equal to its df (Table 6, right-hand section), while the evidence suggesting interactions of treatment with recovery stratum (Table 6, left-hand section) is now less strong. This pattern continues if all zero counts are omitted from the analysis. Residual plots (Fig. 1c and 1d) show slightly more evidence of heavy tails, suggesting that the assumptions of the analysis are less well satisfied.

Estimates, with standard errors, of log (δ_2/δ_1) and log (δ_3/δ_1) are given in Table 7, together with 95% confidence limits for the returns from Treatments 2 and 3 relative to 100 returns from Treatment 1. Again, omission of zero counts has little effect on the estimates. In this example, comparisons between treatments are not changed by omitting zero observations: comparisons between areas and between weeks are, however, seriously altered. With other models for the n_{ij}, elimination of zeroes could also distort estimates of treatment effects.

Negative binomial error

Table 7 shows that replacing the assumption $\sigma^2 = \phi\mu$ for the variance with $\sigma^2 = \mu + \mu^2/k$ makes little difference to the analysis. The difficulty of distinguishing the two variance structures can be seen from the plot of the variances between duplicate catches (Fig. 2).

Normal theory

Analysis of the recovery rate y, when correctly weighted as in Model 3C, gives identical results to the recommended analysis. Analysing y as a normal random variable with constant variance suggests a strong interaction between treatment and region ($F_{6,60} = 32.5, P < 0.01$), but both a graph of variance against mean, like Fig. 2, and the residual plot in Fig. 1b show that the assumptions of the analysis are untenable.

To analyse log (y), we must first add a constant to y to avoid taking the logarithm of zero. The arbitrary choice of this constant renders the procedure of doubtful value. The observed values of y are of the order of 10^{-4} to 10^{-3} so that log $(1 + y)$, a common choice, is little different form $(-y)$.

Aggregating data over regions or weeks

Finally, consider the effect of aggregating the data for analysis, which could here be done in either or both of two ways, over weeks and over regions of tag recoveries. The effects on the model selection process and on estimates of treatment comparisons are shown in the lower parts of Tables 6 and 7. Information is lost on possible interaction between treatments and any factors whose levels have been composited. Even in this example, when such interactions were not found to be impor-

TABLE 7. Estimated comparisons between hatchery treatments based on assumptions of scaled Poisson ($\sigma^2 = \phi\mu$) and negative binomial ($\sigma^2 = \mu + \mu^2/k$) variances.

Data	Variance structure	$\log(\delta_2/\delta_1) \pm$ SE	95% CI δ_2/δ_1	$\log(\delta_3/\delta_1) \pm$ SE	95% CI δ_3/δ_1
Disaggregated	$\phi\mu$	0.376 ± 0.114	(116, 182)	-0.566 ± 0.144	(43, 75)
	$\mu(1 + \mu/k)$	0.396 ± 0.123	(117, 189)	-0.495 ± 0.144	(46, 81)
Disaggregated (zeroes ignored)	$\phi\mu$	0.360 ± 0.101	(118, 174)	-0.572 ± 0.126	(44, 72)
	$\mu(1 + \mu/k)$	0.364 ± 0.105	(117, 177)	-0.558 ± 0.130	(44, 74)
Aggregated over weeks	$\phi\mu$	0.450 ± 0.109	(127, 194)	-0.339 ± 0.138	(54, 93)
	$\mu(1 + \mu/k)$	0.453 ± 0.110	(127, 195)	-0.338 ± 0.134	(55, 93)
Aggregated over regions	$\phi\mu$	0.438 ± 0.143	(117, 205)	-0.534 ± 0.179	(41, 83)
	$\mu(1 + \mu/k)$	0.473 ± 0.169	(115, 223)	-0.419 ± 0.184	(46, 94)
Aggregated over both	$\phi\mu$	0.436 ± 0.148	(116, 207)	-0.359 ± 0.187	(48, 101)
	$\mu(1 + \mu/k)$	0.437 ± 0.152	(115, 208)	-0.364 ± 0.170	(50, 97)

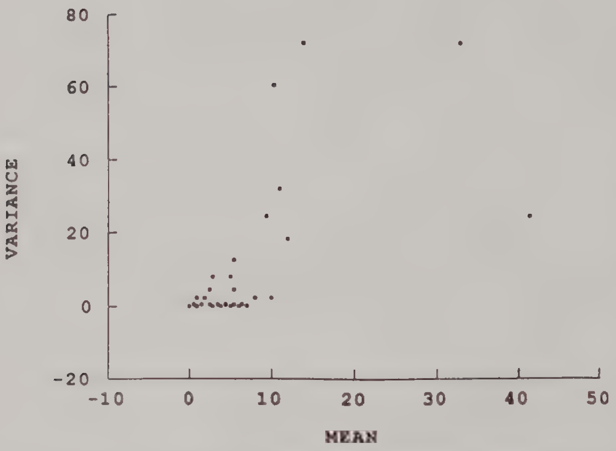

FIG. 2. Plot of variance against mean for the duplicate counts in Clark and Bernard (1987) coho data.

tant, major distortions are introduced into the results. Aggregation over weeks has little effect on model selection with no evidence in Table 6 of either interaction (between treatment and area) or lack-of-fit. By contrast, aggregation over regions leads to a residual χ^2 which is twice its df and to a comparable increase in the interaction deviance (between treatment and weeks). Substantial overdispersion has been introduced. This is shown by the increased standard errors and wider confidence intervals for treatment comparisons given in Table 7. Although our models allow for overdispersion, the particular estimates in Table 7 that would be quoted would depend on the particular form of variance function chosen, with little evidence for selecting one rather than the other. There seems no pattern in the estimates of hatchery differences between disaggregated and aggregated data analyses. Estimates for $\log(\delta_2/\delta_1)$ are roughly the same for all aggregated analyses, higher than for disaggregated, while estimates for $\log(\delta_3/\delta_1)$ are markedly increased by aggregation over weeks but not by aggregation over regions.

Aggregating over codes

If only a single tag code per hatchery had been used, no information would have been available on the adequacy of the most general model: the 60 residual df from the full model disappear. There is no information on whether an observed difference between hatcheries or fisheries represents a real difference in mean level or a lack of independence among fish from the same hatchery. The deviances from any simpler model using data aggregated over codes are identical to the deviance differences between the simpler and the full model (i.e. the tests of interaction in Table 6) using the unpooled data. No new analysis is needed. However, the χ^2 values are changed. In the example, the χ^2 value for Model 4 is 47.4 from the pooled data, less than the interaction value of 60.6 for unpooled data that gave some concern earlier about the selection of that model.

Discussion

The analysis of overdispersed counts is an area of much recent statistical research (Nelder and Pregibon 1987; Morton 1988; Tsutakawa 1988; McCullagh and Nelder 1989; Breslow 1990). The procedure recommended here is possibly the simplest to implement and understand because of its close correspondence to analysis of variance. Analysis of a negative binomial form with variance $\sigma^2 = \mu + \mu^2/k$ can readily be implemented by the iterative GLIM macros of Breslow (1984) or Bennett (1988). In this, as in the proportional Poisson form $\phi\mu$, the overdispersion parameter k is consistently estimated by the moment estimator obtained by dividing the Pearson χ^2 by its df. The question of how best to estimate ϕ or k is still open, since the estimator obtained by dividing the residual deviance D by its df, though inconsistent, has smaller mean square error than the $\hat{\phi}$ used in this paper.

The method of quasi-likelihood (QL) was introduced by Wedderburn (1974) as an extension of the method of least squares to the case where the variance is not constant but constant times a known function $\sigma^2(\mu)$ of the mean μ. A brief introduction to QL, and a clear statement of its large-sample distributional properties, is given in McCullagh (1986) with a more detailed discussion in Chapter 9 of McCullagh and Nelder (1989). Many of the commonly used variance functions $\sigma^2(\mu)$ are properties of standard probability distributions (e.g. $\sigma^2(\mu) = \mu$ for Poisson). In these cases, QL estimates are identical to the maximum likelihood estimates which would be obtained from that probability distribution, and deviance differences are -2 times log likelihood ratios. However, the QL estimators and tests have desirable asymptotic properties without any assumption about the probability distribution of the observations.

An extended study by Breslow (1990) compares the various possible tests for model selection when the true form of the observed overdispersion is both known and unknown. In the latter case, he finds that the procedure recommended in this paper is too liberal, rejecting the null hypothesis when true more often than the presumed α-level. In Breslow's examples of testing for significant differences between medical treatments, a liberal test does seem undesirable. The same considerations may apply when the main aim of the tag–recovery experiment is to compare hatchery treatments: we may not wish to assert the existence of treatment differences when they do not exist. But, for comparing different regions or time periods for the recovery process, it seems more reasonable to think that real differences do exist and that a simpler model is being sought for ease of interpretation and reduction of variances, and we may wish to be more sure than the test indicates that the simpler model is acceptable before using it. If overdispersion is observed, Breslow recommends the use of an empirical score test which approximates the correct α-level but which suffers from severe loss of power, particularly in experiments with few replicates and large α. Thus, this test also leads to retention of the simpler model when a more complex pattern of recovery rates is actually occurring.

Information about overdispersion comes from examination of the residuals from the full model. Without the use of more than one tag code per treatment, no such information is available. When treatments within a hatchery are to be compared, careful consideration needs to be given to the experimental design, including to which groups of fish different codes should be applied. Nonindependence or heterogeneity, both of which may be manifest in overdispersion, can invalidate the results of any experiment. In practice, neither the form of the variance function nor the existence or magnitude of interactions between the factors in the experiment is known. Uncertainty in both mean structure and variance structure makes selection of a valid model from data extremely difficult. Aggregation can induce or mask the appearance of interactions, as demonstrated in the example.

All the arguments, from statistical theory and fisheries methodology, support the principle of keeping overdispersion as low as possible by analysing the data at the most disaggregated level possible.

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A Reliable Tag–Recapture Technique For Estimating Turbine Passage Survival: Application to Young-of-the-Year American Shad (*Alosa sapidissima*)

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A new technique (HI-Z Turb'N Tag, U.S. Patent No. 4,970,988) for estimating turbine passage survival was applied to juvenile American shad (*Alosa sapidissima*) under three operating conditions at a hydroelectric project. Fish are fitted externally with the Turb'N tag and introduced into turbine penstocks. The Turb'N Tag inflates after turbine passage and buoys fish to the surface for recapture and examination; after removal of tags, fish are held to assess long-term effects. Almost all (96%) test (299) and control (300) fish were recovered; average recovery time was less than 9 min. The overall short-term (1 h) survival of test fish, adjusted for control, was 97%; 24- and 48-h survivals were 98 and 94%, respectively. The 48-h survival of test fish was 98–100% for mixed flow and Kaplan turbines and 66.8% for the mixed flow unit in the vented mode. Acute control mortality was negligible (<5%). Our technique offers several significant advantages over traditional net recapture methods: applicable to wide range of species and size; allows predetermination of statistically valid sample size, level of significance, and power of the test to determine need for mitigation measures; and estimation of cumulative effects of multiple turbine exposure.

Une nouvelle technique (étiquette HI-Z Turb'N, brevet américain n° 4,970,988) pour estimer le taux de survie au passage en turbine a été appliquée à de jeunes aloses savoureuses (*Alosa sapidissima*) sous trois régimes de fonctionnement dans une installation hydroélectrique. On fixe sur les poissons, de façon externe, l'étiquette Turb'N, et on les introduit dans les conduites forcées. Après le passage dans la turbine, l'étiquette Turb'N se gonfle et amène le poisson à la surface où on peut le capturer et l'examiner; après prélèvement des étiquettes, on garde les poissons pour évaluer les effets à long terme de l'opération. Presque tous les poissons sujets de l'expérience (299) et témoins (300) ont été récupérés (96 %); le temps moyen de reprise était inférieur à 9 min. Le taux global de survie à court terme (1 h) des poissons soumis à l'expérience, après rajustement en fonction des témoins, était de 97 %; les taux de survie après 24 et 48 h étaient respectivement de 98 et 94 %). Le taux de survie des poissons était de 98 à 100 % pour les turbines mixtes et Kaplan et de 66,8 % pour l'unité mixte avec prise d'air. La mortalité aiguë chez les poissons témoins était négligeable (<5 %). Notre technique offre plusieurs avantages importants par rapport aux méthodes traditionnelles de recapture au filet : elle s'applique à des espèces et des tailles très diverses; elle permet de prédéterminer la taille d'échantillon valide sur le plan statistique, le niveau de signification et la puissance du test pour savoir s'il est nécessaire de prendre des mesures d'atténuation; de plus, elle permet d'estimer les effets cumulatifs de passages multiples en turbine.

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The surge in developing hydro sites combined with a strong impetus to preserve or restore migratory fishes (salmonids and clupeids in particular) has elevated fish mortality by turbine passage to a major concern. Numerous reviews on turbine-related fish mortality (Ruggles 1980; Bell 1981; Turbak et al. 1981; Monten 1985; EPRI 1986; Eicher Associates, Inc. 1987; Ruggles and Palmeter 1989; Ruggles et al. 1990) indicate that most effort has been expended on protection, exclusion devices, and evaluation of mortality of salmonid smolts. Eicher Associates, Inc. (1987) concluded that in spite of decades of research on salmonids, much uncertainty remains in estimating turbine-related mortality. A variety of factors may cause this uncertainty; namely, design of turbine, wicket gate setting, head, species of fish examined, size of fish, rotation of turbine blades, turbine blade angle, and number of blades. These factors in combination with uncertainties associated with conventional tag–recapture techniques make results of turbine-induced mortality studies difficult to interpret. In many cases, however, expensive mitigation measures (spillage, bypass structures, screens, etc.) have been suggested, initiated, or installed prior to actually measuring turbine-passage mortality. An equally important issue is the potential for these mitigation measures to inflict significant mortality or injury relative to passage through hydraulic turbines.

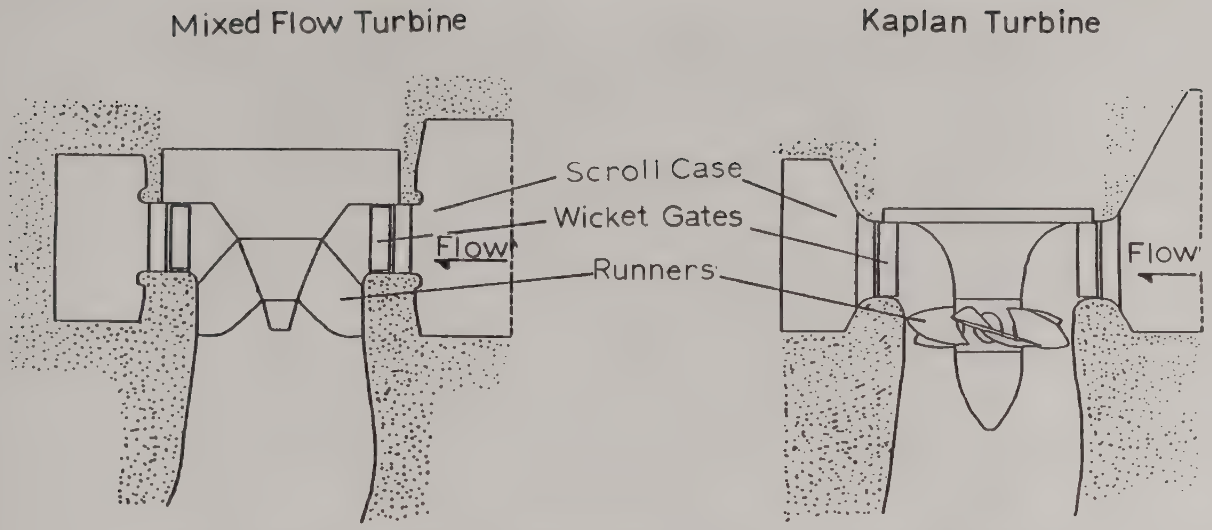

FIG. 1. Schematics of the Kaplan and mixed flow turbines at the Safe Harbor Hydroelectric Station.

Within the last decade, attention has also focused on the need for mitigation of potential losses of alosids during turbine passage (Gloss et al. 1982; Kynard et al. 1982; Davies 1988; Ruggles et al. 1990; Stokesbury and Dadswell 1991). Alosids have been targeted for restoration in many East Coast rivers. Although upstream passages have been provided for alosids at many hydroelectric projects, turbine entrainment mortality of emigrating juveniles remains a serious concern.

Major obstacles block the performance of satisfactory turbine passage mortality studies on juvenile alosids. Alosids are extremely sensitive to handling and tagging; thus, control mortality (in mark–recapture studies) has been high (Ruggles et al. 1990). The inability of investigators to satisfactorily separate the collection mortality from that due to turbine passage and avoid fish extrusion through nets further compounds the problems of reliable mortality estimation. Ruggles and Palmeter (1989) concluded from a netting study of juvenile alewife (*Alosa pseudoharengus*) that total fish recapture after turbine passage is the most effective experimental design to gather unambiguous results quickly. Burnham et al. (1987) also discuss the analytical advantages of high recapture rates of fish in reliably estimating turbine-related mortality of fishes at hydroelectric projects. However, capturing all test and control juvenile alosids has proven difficult.

Our objective is to show the application of the HI-Z Turb'N Tag and recapture method (U.S. Patent No. 4,970,988) in reliably estimating both the short-term (1 h) and long-term (48 h) turbine passage survival rates for juvenile American shad (*Alosa sapidissima*) (90–140 mm fork length (FL)) at the Safe Harbor Hydroelectric Station, a 417-MW peaking hydroelectric project on the lower Susquehanna River (river km 49). The need for rapid recapture of a high proportion of the test and control tagged fish coupled with low handling mortality was inherent in fulfilling the recommendations made by Ruggles and Palmeter (1989).

Methods and Materials

The Safe Harbor Hydroelectric Station consists of a concrete gravity dam 1484 m long and 23 m high, with 12 generating units. The project has seven Kaplan and five mixed flow turbines (Fig. 1) for a total hydraulic capacity of 3143 m^3/s. Each turbine is capable of passing approximately 245 m^3/s. The Kaplan turbines have five blades and are 5.6 m in diameter with a runner speed of 109 rpm. The mixed flow turbines have seven blades, a diameter of 6.1 m, and a runner speed of 77 rpm. Survival of young American shad was estimated at both mixed flow and Kaplan turbines. The mixed flow turbine was tested under vented mode (aeration of flow to increase dissolved oxygen) and unvented operating mode.

Collection and Handling of Specimens

Young-of-the-year American shad were collected by a specially designed lift net from the forebay of the Holtwood Hydroelectric Station, 11 km downstream from the study site. This net minimized handling effects because after lifting, shad were directly transferred in water to transport tanks. Fish were transported to the study site in sealed 75-L circular tanks filled with a 0.5% solution of sodium chloride (NaCl) and river water. The salt solution reduced osmotic and ionic imbalance due to handling stress (Meinz 1978). To the extent possible, shad were not removed from the water during handling. Upon arrival at the study site, fish were transferred to 950-L circular holding tanks positioned at the station headworks and held for 24 h prior to testing. These holding tanks were continuously supplied with river water. The dissolved oxygen of the water remained $\geqslant$9 mg/L and water temperature ranged between 10.0 and 23.0°C.

Specimens were removed from the holding tanks in water-filled buckets and placed into a 75-L circular tub prior to tagging and release. Initially, juvenile shad were held in fresh river water, but experience showed that keeping juvenile shad in a 0.5% salt solution for up to 2 h prior to tagging reduced handling stress (see later). Subsequent tagging was, therefore, conducted after shad had been held 0.5–2 h in the salt solution.

Circular net pens for assessment of delayed mortality of recovered test and control fish were located in an eddy 61 m downstream of the powerhouse to minimize the influence of extraneous factors (i.e. net impingement, human disturbance). The net pens were 1.5 m in diameter, 1.2 m deep, and covered on the bottom, sides, and top with 7-mm rigid plastic netting.

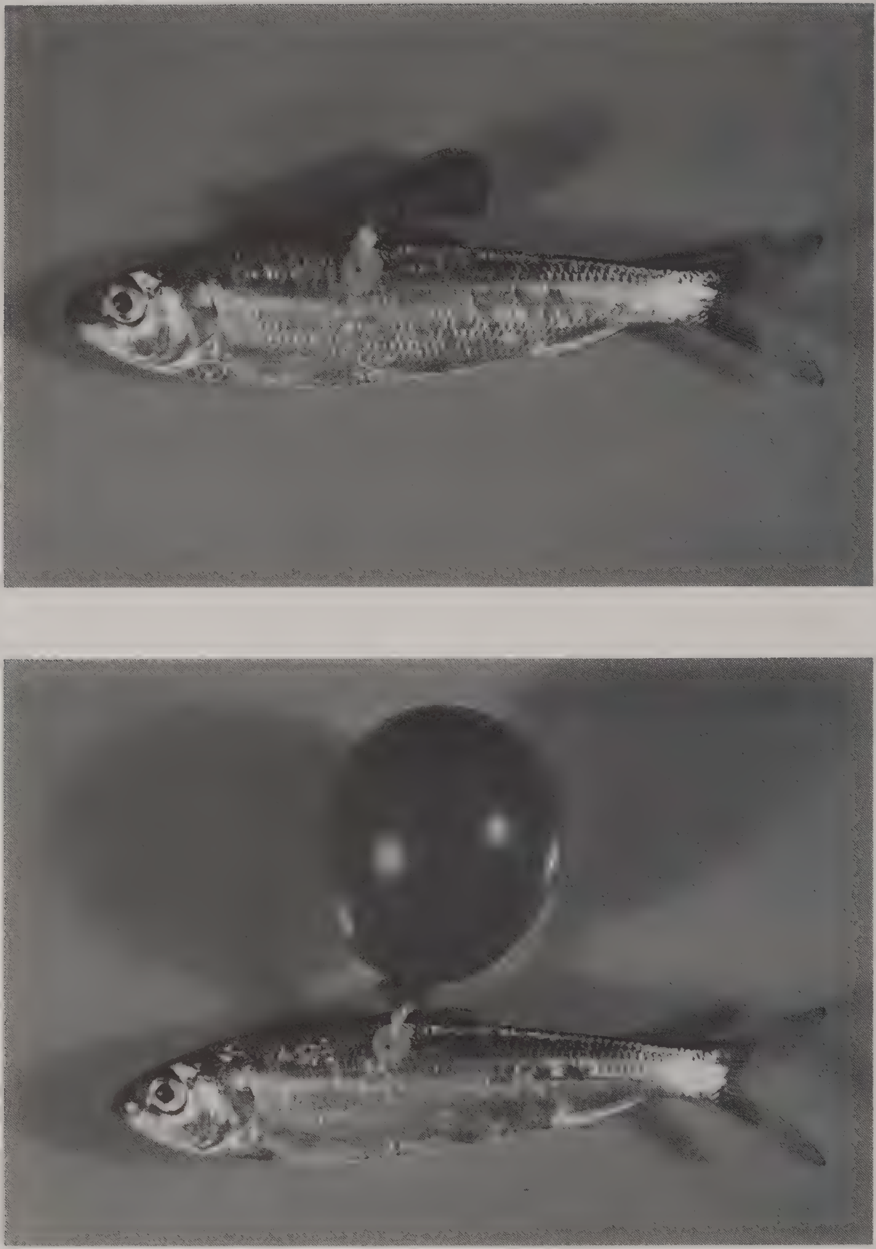

FIG. 2. Uninflated and inflated Turb'N Tag.

Tagging and Induction of Fish

The Turb'N Tag recovery technique (a balloon-type tag) is chemically based and involves external attachment of a small uninflated Turb'N Tag to each fish (Fig. 2). Prior to release of a fish, the tag is activated by injecting 1 mL of water to initiate the chemical reaction. The delayed chemical reaction releases a gas and inflates the tag. Shortly after turbine passage the tag slowly begins to inflate, buoying the fish to the surface for recapture (Fig. 2). The tag inflation time can be adjusted to between 2 to 60 min depending on the water temperature and configuration of the study site. Once recaptured, the inflated tag is removed from the fish with a modified plier or clip (generally within seconds), keeping the fish immersed in water, and the fish's condition can be carefully determined. The fish is then transported in buckets filled with water to a holding pen for assessment of long-term effects. Because of the short duration (few minutes) that the tag remains on the fish, no adverse effects on fish behavior are encountered. Fish tagged and kept in holding pools showed over 80% survival with no apparent effects on swimming ability after tag removal (Mathur and Heisey 1992). Generally, anesthetic is not necessary for tag application on small fish but may be useful on large active fish

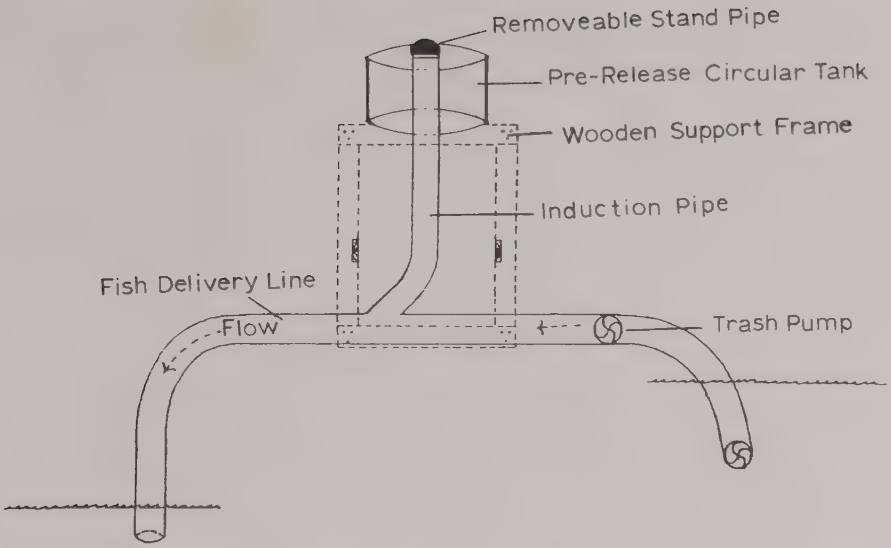

FIG. 3. Schematic of the fish induction system used for the turbine passage survival study of juvenile American shad.

to reduce tagging and handling stress. The tag has been successfully field tested on fishes ranging approximately from 60 to 400 mm in total length (Mathur and Heisey 1992). Larger specimens (>200 mm) may require more than one tag to buoy them.

For this study, each juvenile shad was individually removed from the holding tanks in a water-filled bucket and externally fitted with a Turb'N Tag and a neutrally buoyant miniature radio transmitter. A tag and transmitter were attached by a stainless steel pin (5 or 10 mm long) through the anterior dorsal musculature so that they trailed on either side of the fish. The pin was inserted with a modified ear-piercing gun and secured by a small plastic disc. Attachment of both types of tags increased the probability of recovery if the fish moved rapidly out of the tailrace and could not be located visually. However, it may not be necessary to utilize miniature radio tags for studies at hydroelectric projects with low-volume discharges or if fish size becomes limiting. Clupeids less than 90 mm FL generally do not respond well to attachment of miniature radio tags. Other species more resistant to handling can accept miniature radio tags (Mathur and Heisey 1992). However, if radio tags are needed, they can be reused upon recovery of each fish.

Uninflated Turb'N Tags were made of bright-colored latex, pear-shaped with dimensions of approximately 38 × 13 mm; each weighed 1.5 g. Upon full inflation, the tags measured 75 mm long by 50 mm in diameter. The radio tags weighed 1.7 g, were approximately 10 × 30 mm, and propagated signals through a 27-cm thin wire antenna.

Prior to release of each fish the tag was activated by injecting 1 mL of water to initiate the chemical reaction. Tagged fish were introduced individually into the penstock of an operating turbine at a depth of approximately 10 m by an induction apparatus consisting of a small holding basin attached to a 7.6-cm-diameter reinforced plastic delivery line (Fig. 3). A gasoline-powered trash pump continuously supplied water to the system to ensure rapid transport of fish through the delivery line within a continuous flow of water. Control fish were tagged and released individually through the same induction apparatus directly into the tailrace to separate the effects of tagging/handling from those due to turbine exposure (Fig. 4). Similar sized fish were used for test and control releases.

Fish Recapture

Test and control fish were recaptured by homing on a radio signal or visually spotted by the inflated tag. Each buoyed fish was retrieved by using a 11-L bucket. Fish that failed to surface immediately were monitored via radio signals. Recapture time for each tagged fish was recorded.

The recaptured test and control fish were placed in an onboard holding tank, carefully examined for mechanical injury, tags and attachment pin removed, and tagging site examined. Fish were then transferred to floating net pens for long-term observation (48 h). Net pens were checked for both test and control fish mortalities at 24 and 48 h. Fish were classified into (1) short-term survival or mortality (≤1 h), (2) not recovered and unknown status, and (3) long-term survival or mortality (≤48 h).

Sample Size and Statistical Analysis

Because the analysis of survival experiments based on tag–recapture data is a function of the total number of recaptured fish, statistical significance, and the power of the test, we used the results of a pilot turbine passage survival study conducted at the site in 1989 to calculate the sample size necessary to detect a desired level of statistical difference between survival of control and test fish for this study. To detect a statistical difference in short-term turbine-related mortality of >0.10 at ordinarily accepted levels of alpha ($\alpha = 0.05$) and statistical power ($1 - \beta = 0.8$), we assumed a control survival of 95% and recapture rate of 90%. We estimated that a release of 100 juvenile shad each for test and control was needed for each operating condition. Sample size requirements for other combinations of expected recapture rates, control mortality, and statistical significance were also calculated. However, the present paper deals only with the above-stated statistics.

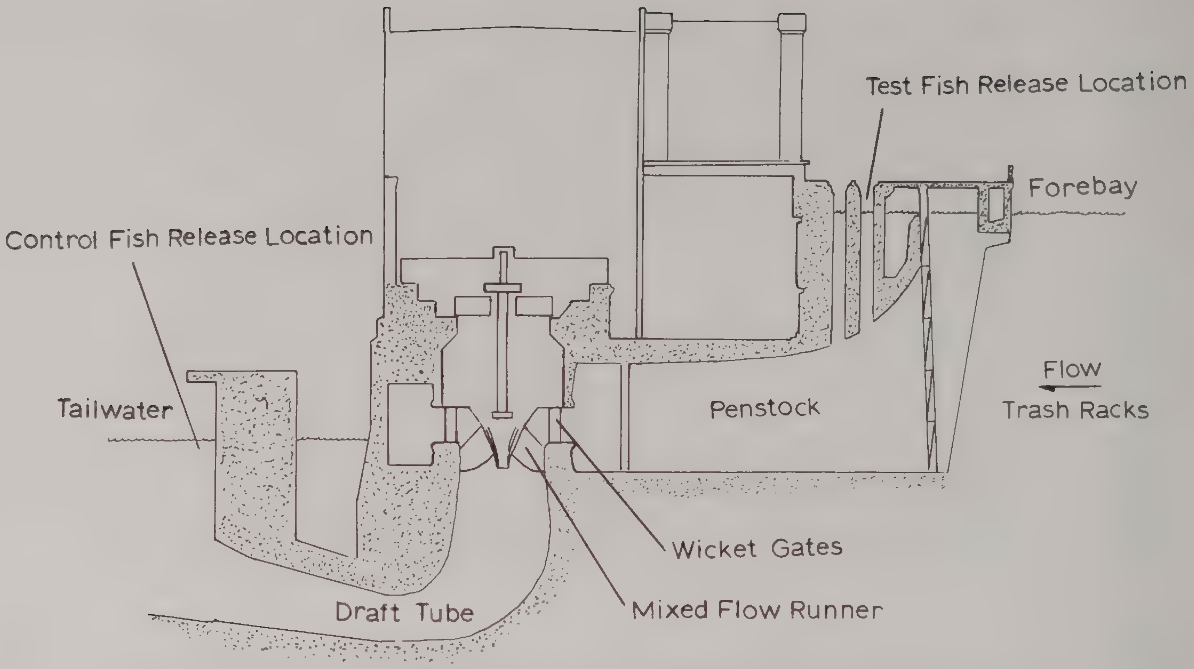

FIG. 4. Release locations of test and control juvenile American shad for the turbine passage survival study.

The short-term (≤1 h) and long-term (24 and 48 h) turbine-related mortality (relative risk) of juvenile American shad was calculated according to the equation given in Fleiss (1981):

$$P_e = \frac{P_2 - P_1}{1 - P_1}$$

where P_e = turbine-related mortality adjusted for control, P_1 = fractional mortality of control fish, and P_2 = fractional mortality of test fish.

The confidence intervals on the estimated turbine-related mortality (P_e) were calculated using the formula for standard error (SE) given in Fleiss (1981):

$$\text{SE of } P_e = \left(\frac{1}{Q_1}\right)\sqrt{\frac{P_2Q_2}{N_2} + (1 - P_e)^2\left(\frac{P_1Q_1}{N_1}\right)}$$

where P_1 = fractional mortality of control fish, P_2 = fractional mortality of turbine-exposed fish, Q_1 = fractional survival of control fish, Q_2 = fractional survival of turbine-exposed fish, N_1 = sample size of control fish, and N_2 = sample size of turbine-exposed fish.

For the purposes of these analyses, fish categorized as "unknown" or "tag and pin" or preyed upon were conservatively considered as dead.

The significance of differences in recapture and survival rates were statistically determined by the Fisher's exact chi-square 2 × 2 contingency test of independence (Sokal and Rohlf 1981). Data were analyzed using the Statistical Analysis System (SAS Institute, Inc. Version 6.03).

Results

Recapture Rates

The recapture of test and control fish was high with an overall rate of 96% (Table 1). Although we recaptured similar ($P > 0.05$) proportions of control and test fish, slight differences (nonsignificant) were observed between test conditions. We recaptured 92% of both the test and control fish from the mixed flow unit in unvented mode and 97–99% from the other test conditions). The seemingly higher proportion (8%) of "unknown" test and control fish for the mixed flow unit in unvented mode appeared to be due to predation by walleye (*Stizostedion vitreum*) on juvenile shad. Predation was confirmed because in one case the tag inflated while the young shad was being swallowed, bringing the walleye to the surface.

The recovery times were short, averaging less than 9 min under all test conditions (Table 1), and similar ($P > 0.05$) both between operating conditions and between control and test fish.

Short-Term Survival

Short-term survival of test fish was high and showed little variation (Table 2). Overall survival was 97%, and differences in survival were not significant ($P > 0.05$) between turbines or between control and test fish.

The high recapture rate of live test and control specimens provided a relatively large sample size for assessment of long-term survival (Table 3). The 24-h survival of juvenile shad at all three operating conditions ranged from 97 to 100%. The 48-h survival of juvenile shad at the mixed flow turbine in unvented mode and Kaplan unit ranged from 98 to 100%. The 48-h survival of juvenile shad at the mixed flow unit in vented

TABLE 1. Comparative statistics on recovery rates and times of test and control young American shad released for the turbine passage survival study under three turbine operating conditions (percentages in parentheses).

	Mixed flow (unvented)		Kaplan		Mixed flow (vented)		Total	
	Control	Test	Control	Test	Control	Test	Control	Test
No. introduced	100	100	100	100	100	99	300	299
No. recovered/ known status	92 (92%)	92 (92%)	99 (99%)	99 (99%)	98 (98%)	96 (97%)	289 (96%)	287 (96%)
Recovery time (min)								
$\bar{X}$	8.9	6.7	4.5	5.4	8.3	8.7	7.2	6.9
SE	1.1	0.4	0.3	0.4	0.8	0.5	0.5	0.3
Min.	1	1	1	1	1	2	1	1
Max.	13	35	78	17	65	25	78	25

TABLE 2. Comparison of short-term (1 h) turbine passage survival of young American shad released under three turbine operating conditions. Test fish survival rate adjusted for control mortality. Fish of unknown status included with dead.

	Mixed flow (unvented)	Kaplan	Mixed flow (vented)	Total
No. introduced	100	100	99	299
No. survived	90	97	92	279
% survival	97.8	98	98.9	96.9
95% CL[a]	93.4–100	94.1–100	94.3–100	93.2–100

[a]Upper limits truncated at 100%.

mode was 67%, with a significantly higher survival of control fish ($P < 0.05$). However, these differences are considered suspect and unreliable due to a high mortality of both test and control fish caused by natural meteorological events. Two weeks of sustained high natural river flows (6000 m^3/s) and falling air temperatures lowered water temperature (from 22.5 to 9.0°C) and created excessive turbidity. Survival of control fish at 24 and 48 h was 72% (71 of 98 held) and 54% (53 of 98), respectively. The respective test fish survival rates unadjusted for control at 24 and 48 h were 74% (68 of 92) and 38% (35 of 92).

Injury

Only 5% of the 283 test fish and 2% of the 289 control fish exhibited external injury marks or scale loss. The primary injury to test fish was small lacerations. Control fish showed minor hemorrhaging or bruises.

Discussion

A combination of high recapture rate and short recapture time indicates that the Turb'N Tag technique worked well and matched the initial expectation. The near 100% survival of control fish, particularly short term, fulfilled the criterion set forth in the experiment and allowed reliable estimation of turbine-related mortality. Burnham et al. (1987) stated that the reliability of turbine passage survival estimation is substantially enhanced when investigators can recover a high proportion of marked fish. Past studies on juvenile alosids have been hampered by generally low recovery rates and high net induced mortality due to abrasion or extrusion through nets. The present recoverable tag overcomes many of the logistical problems associated with sampling in turbulent conditions at hydroelectric projects and was able to provide a more realistic estimate of turbine-related mortality of juvenile shad.

The high rates of recapture of live fish provided an opportunity to assess long-term mortality. However, the tag removal technique on the recovered fish and holding of some fish in freshwater prior to tagging caused high long-term mortality of both control and test fish at the mixed flow unit (unvented mode). On the first day of testing, young shad were held in freshwater and the tags from the recovered fish were removed by needlenose pliers. The 24-h mortality was 56 and 78% for the control and test fish, respectively. At 48 h the mortality was 83% in control and 82% in the test group. Subsequently, young shad were held in 0.5% NaCl solution prior to tagging (see Methods and Materials section). The 24-h mortality decreased substantially, but the condition of the fish at 48 h indicated that the tag removal was still too stressful. Consequently, the tag removal procedure was modified by using two "clips" to pull the plastic disc off the attachment pin that secured the tags to the fish. These modifications resulted in 24-h survival of 100% for control fish. Other studies show that these modifications have improved the procedure considerably and appear to be applicable to a wide range of species and sizes (Mathur and Heisey 1992). The smallest clupeid tagged with the Turb'N Tag was 80 mm total length and the largest fish, a smallmouth bass (*Micropterus dolomieu*), tagged to date measured 305 mm.

There are three principal risks associated with turbine passage: direct strike by the rotating turbine blades, rapid change in water pressure, and hydraulic shear forces. These risks, however, are not universally applicable to all species and their life stages at hydroelectric projects. Unless an individual fish is physically retrieved for careful examination, it may be difficult to estimate these risks quantitatively. Our technique allowed quantification of these risks. The probability of direct contact of fish with the rotating blades is primarily a function of fish morphology and size, turbine runner speed, turbine blade clearance, wicket gate clearances, turbine type and orientation, and hub diameter (Monten 1985). A higher runner speed, larger fish size, and narrower blade clearances increase the probability that an entrained fish will be struck by the turbine blade.

The near absence of mechanical injury to juvenile American shad in the present study indicated that direct contact with the rotating turbine blades was a low probabilistic event under the operating conditions that were tested at Safe Harbor. This may

TABLE 3. Comparison of long-term (24 and 48 h) turbine passage survival of juvenile American shad released under three turbine operating conditions. Fish lost from net pens due to high flow excluded from calculations.

	Mixed flow (unvented)[a]	Kaplan	Mixed flow (vented)[b]	Total
		Test fish		
No. held	90	97	92	279
No. alive (24 h)	63	97	68	228
Adjusted survival (%)[c]	100	100	97	98
No. alive (48 h)	52	85	35	172
Adjusted survival (%)[c]	100	98	67	94.3
		Control fish		
No. held	92	99	98	289
No. alive (24 h)	63	99	71	233
% alive (24 h)	68	100	72	81
No. alive (48 h)	47	83	53	183
% alive (48 h)	53	98	54	67

[a] Twenty test and 20 control fiush held in freshwater prior to testing; tags from 70 test and 70 control fish removed without using the modified "clip."

[b] Five test and five control fish held at a water temperature of 21°C; remaining fish (87 test and 93 control) held at a water temperature of 9–11.0°C.

[c] Test fish survival adjusted for control mortality (see text).

be due to the slow runner speed and wide clearances between turbine runner blades. Cada (1990) suggested that most of the factors that result in mortality at low head hydroelectric projects can be attributed to direct blade contact, and little mortality is caused by turbulence, cavitation, or pressure changes.

Young American shad were subjected to little pressure change (head at Safe Harbor is approximately 18 m) during turbine passage. At low head projects (<30 m), pressure change is usually of no consequence because relatively little change in pressure occurs; fish passage is virtually instantaneous. Another study conducted at a hydroelectric project (net head of 29 m) on the lower Susquehanna River similarly showed no visible effects of pressure on juvenile shad (RMC Environmental Services, Inc., unpubl. data). However, these results are contrary to those of other investigators who have implicated pressure-related injuries to alosids at projects with a net head of even less than 15 m (Gloss et al. 1982; Kynard et al. 1982; Stokesbury and Dadswell 1991). We can only hypothesize on the possible causes of differences. The most likely causative agent for the higher rate of injury appears to be the sampling gear, namely, the recapture netting system. Ruggles et al. (1990) have pointed out several problems with netting studies, one of which is high injury rates. Test fish are frequently impinged on the nets or abraded against the nets resulting in injuries or mortalities resulting in high estimates of turbine-related mortality. Because nearly every tagged fish was recovered individually in the present study, it allowed for the careful examination of each fish for location of mechanical injury and scale loss. Previous studies lacked the capability to unequivocally separate turbine-induced injury/mortality from that due to handling/tagging and netting.

The low estimated mortality of juvenile shad at the Safe Harbor Project is in contrast with that reported in the literature for young alosids exposed to Kaplan and other types of turbines. Previous studies, mostly limited to estimation of acute effects of turbine passage, have been plagued by high control mortality, lack of a true control, methodological problems, inadequate sample size, and low recapture rates (Turbak et al. 1981; Gloss et al. 1982; Kynard et al. 1982; Ruggles et al. 1990). Kynard et al. (1982) reported turbine passage mortality rates of 62–82% for juvenile shad and blueback herring (*Alosa aestivalis*) (60–90 mm) at the Hadley Falls Station (Kaplan turbine) on the Connecticut River. These mortality estimates appear intuitively too high and in conflict with findings on larger fishes. Bell and Kynard (1985) estimated mortality of postspawned adult shad (450–600 mm) to be less than 15% (when adjusted for control mortality). The probability that entrained juveniles will be struck by a turbine blade is much less than for the adults; thus the mortality for larger fish should be higher. Interestingly, mortality of Atlantic salmon smolts (*Salmo salar*) (210–360 mm) was calculated to be less than 7% (when adjusted for control) at the Hadley Falls Station (Kynard et al. 1982); again the salmon smolts were much larger than the juvenile clupeids tested. It is interesting to note that the turbine-related mortality of larger fishes exposed to the Kaplan turbine is somewhat similar to the low mortality reported herein for juvenile American shad. Kynard et al. (1982) used a netting recovery system placed approximately 300 m downstream of the powerhouse for the juvenile clupeid study, while studies on adult shad and salmon smolts utilized radio tags. Recapture rates of marked fish were low (<15%). However, radiotelemetry techniques allowed these investigators to know the status (live or dead) of a high proportion of test fish. Ruggles et al. (1990) noted differences between mortality of naturally entrained young alewife (14%) and those force-fed into the tube turbine (44%). Virtually zero acute control mortality coupled with nearly 100% recovery rates within minutes in our study provided a reliable separation of turbine-related losses from those due to handling stress, rendering interpretation and statistical analysis of the data straightforward.

Evidence is mounting that turbine-induced injury/mortality represents a discriminate stress to the exposed population. Hence, chances of long-term survival for fish that survive passage through turbines at low head projects are as good as those not exposed to turbines (Ruggles 1985). Nearly 100% survival of juvenile shad at 24 and 48 h after turbine passage in most cases of the present study supports this hypothesis. The lower 45-h survival at the mixed flow turbine in vented mode most likely resulted from low water temperature and high turbidity. Chittenden (1971, 1972) reported that young shad are difficult to maintain at low water temperatures and become lethargic. Water temperature was <11.0°C when the test at the mixed flow unit in vented mode was conducted. Many shad held at this temperature appeared more lethargic than those held at higher water temperature. Low water temperatures generally coincide with the end of the juvenile emigration period and many physiological changes, preparatory to the seaward journey, have already taken place. Emigrating young shad may be more susceptible to handling at this time (Howey 1985).

Our data indicate that juvenile American shad may not be as susceptible to injury/mortality during their emigration through turbines as might have been perceived previously and may in fact withstand multiple turbine exposures. All the juvenile shad utilized in our tests had already passed one of the turbines at Safe Harbor Project; these fish were collected for the study from the forebay of the next hydrostation downstream. No spill events occurred during the period when these fish were moving downstream, so they must have passed through turbines. The condition of the recovered shad indicated that they could have been retagged and released again through turbines. Although not part of the present study the ability to recapture live fish may offer opportunities to evaluate the cumulative effects of multiple turbine passage or the physiological and behavioral responses of fishes.

The superiority of complete recapture of tagged fish in release–recapture survival experiments has some significant practical implications. An adequate sample size can be established, correct statistical models applied, and fewer assumptions made. Experience indicates that the importance of appropriate sample size, power of the test, statistical model, and assumptions for quantifying effects on fishes is frequently underestimated (Burnham et al. 1987; Peterman 1990). In the present case, results of a small pilot study provided information on the expected recapture rates and control mortality to calculate a statistically valid sample size to detect a specified magnitude of difference with a given level of significance and power. The resulting small number of fish utilized in our study is in contrast with the need for marking large numbers of fish (several thousands or even millions to ensure a high number of recaptures for valid statistical analyses) in a conventional mark–recapture (i.e. staining, fin clipping, etc.) study using elaborate recapture devices and including many assumptions. The efforts required to collect and recover a large number of specimens substantially increase complexity and cost of a turbine passage survival study. Scarcity of specimens in rivers targeted for fish restoration can virtually eliminate the possibility of conducting a statistically reliable mark–recapture experiment. In the Susquehanna River, the only sources of young American shad are hatchery releases of 18-d-old fry and a small number of fingerlings. Natural reproduction is limited to the progeny of adults transported upstream by a trap and transport program at Conowingo Dam. Historically, adequate numbers of young shad have not been available in the Susquehanna River for conventional mark–recapture turbine passage survival studies.

Unreliable estimates of fish losses or the preconceived notion that turbine-related mortality is nearly 100% have hampered assessments of true effects of hydroelectric turbines on alosids in the northeastern United States and southeastern Canada. If the turbine-related losses are overestimated or perceived to be high, there may be demand for alternative routes (diversion devices, spills, screens, etc.) for fish moving downstream where none may be needed for successful propagation of populations. It is logical to expect that any recommended mitigative measure should achieve a level of protection significantly higher than that afforded by turbines. However, Ruggles et al. (1990) cited a study by Dawley et al. (1989), which reported a 14% greater mortality of chinook salmon (*Oncorhynchus tshawytscha*) fingerlings released into the fish bypass system at the Bonneville Dam Second Powerhouse, Columbia River, than fish passing through the turbines. The Bonneville Dam Fish Bypass System was considered the state-of-the-art fish protection system when constructed in the early 1980's. Apparently, these results suggest that little overall enhancement is afforded to downmigrants by the bypass system.

If the turbine-related losses are underestimated, then protection of the species may be compromised. However, without reliable quantitative data, it is impossible to make a rational decision on this important issue. Because mitigative measures are typically expensive and less than 100% effective in protecting outmigrants, it is necessary to reliably estimate turbine passage survival prior to choosing the course of action.

The new tag–recapture technique shows substantial promise in resolving the above dilemma by providing direct reliable estimates of turbine-related injury/mortality. It is hoped that this in turn will improve the quality of predictions in the assessment of actual effects of turbine passage on fish populations. The potential progress of fish restoration programs can be better gauged by fisheries managers and decision makers.

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Feeding, Reconditioning, and Rematuration Responses of Captive Atlantic Salmon (*Salmo salar*) Kelt

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Wild Atlantic salmon (*Salmo salar*) kelt were reconditioned in the laboratory by initiating their feeding during the winter on freshly thawed Atlantic silverside (*Menidia menidia*) supplemented with vitamins and trace minerals. Some kelt improved in condition by April, and by June the majority were reconditioned. Some females skipped a year of reproductive activity with most rematuring a second time the following year. One group of females rematured and was spawned a third time without skipping another reproductive cycle. Plasma levels of vitellogenin, estradiol, and testosterone remained low in reproductively inactive female kelt; in contrast, these substances increased and peaked just prior to spawning in late October in maturing female kelt. In males, plasma levels of testosterone and 11-ketotestosterone rose in conjunction with testicular development, reaching peak hormone values during the period of spermiation. Although good-quality eggs were collected from reconditioned kelt according to high egg fertilization rates and high rates of egg survival through the eyed and hatching stages, most kelt yolksac larvae died just prior to swim-up. High mortality rates for kelt larvae suggest that either the silverside diet is nutritionally deficient or that the physiology of reconditioned kelt broodstock is inadequate for good-quality egg production.

On a reconditionné des charognards sauvages de saumon de l'Atlantique (*Salmo salar*) en laboratoire en commençant à les nourrir en hiver avec des capucettes (*Menidia menidia*) fraîchement décongelées, avec un supplément de vitamines et de minéraux traces. Dans certains cas, l'état des poissons s'améliorait dès avril, et en juin la majorité des charognards étaient reconditionnés. Certaines femelles ont sauté un an d'activités reproductives, mais la majorité a connu une deuxième maturation l'année suivante. Un groupe de femelles a connu pour la troisième fois la maturation et la ponte sans sauter un autre cycle reproducteur. Les concentrations plasmatiques de vitellogénine, d'oestradiol et de testostérone sont restées faibles chez les charognards femelles inactives sur le plan de la reproduction; par contre, les concentrations de ces substances ont augmenté et ont atteint un pic juste avant la ponte, à la fin d'octobre, chez les femelles en maturation. Chez les mâles, les concentrations plasmatiques de testostérone et de 11-cétotestostérone ont augmenté en même temps que le développement testiculaire, pour atteindre un pic de la présence d'hormones pendant la période d'émission du sperme. Bien qu'on ait recueilli des oeufs de bonne qualité chez des charognards reconditionnés, qu'on ait obtenu des taux élevés de fécondation des oeufs et de survie pendant les stades de l'oeuf embryonné et de l'éclosion, la plupart des larves vésiculées sont mortes juste avant l'émergence. Les taux élevés de mortalité chez les larves provenant de charognards semblent indiquer soit que l'alimentation à base de capucettes est carencée sur le plan nutritif, soit que la physiologie des charognards reconditionnés utilisés comme reproducteurs ne permet pas d'obtenir une production d'oeufs de bonne qualité.

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Wild Atlantic salmon (*Salmo salar*) possess a relatively complex life cycle considering the freshwater and seawater phases of their larval, juvenile, and adult development. Indeed, after returning specifically to their river of origin for spawning, postspawned Atlantic salmon kelt are capable of surviving and reconditioning sufficiently to remature and spawn again once or perhaps a number of times (Ducharme 1969; Johnston et al. 1987, 1990; M. Hambrook, pers. comm.). Recently, Gauthier et al. (1989) and Johnston et al. (1990) reported that artificially reconditioned Atlantic salmon are capable of spawning again in the year following their first reproduction in the wild. Our interest was to study the reproductive performance of wild Atlantic salmon kelt reconditioned in captivity. Therefore, we monitored the feeding responses of individual fish as indicated by body condition together with the proportion of kelt undergoing rematuration. Rematuration was monitored by plasma vitellogenin and sex steroid profiles. In addition, kelt egg quality was determined from egg fertilization rates, egg survival rates through the eyed and hatching stages, and the survival of kelt yolksac larvae to swim-up.

Materials and Methods

Source of Kelt

Wild Atlantic salmon grilse from the Indian River, Newfoundland, were initially retained at the Indian Brook Atlantic salmon research station where they were spawned by hand in November 1985. After being overwintered in freshwater at the Black Brook salmon holding facility, the kelt were vaccinated in April (Aquahealth vibriogen, 0.3 mL) and adapted to seawater cages in June and maintained for the summer on a formulated moist pellet capelin diet (Pepper and Parsons 1987). In November 1986, 51 nonmature kelt (43 females and 8 males) were transferred to the Marine Sciences Research Laboratory for holding in freshwater (Experiment 1).

Another 69 wild kelt (56 females and 13 males) were obtained after they had been stripped of eggs and sperm at the Noel Paul Brook (Exploits River, Newfoundland) spawning channel. In December 1987, approximately 1 mo after spawning, these fish were also transported for rearing in either freshwater or brackish seawater to the Marine Sciences Research Laboratory (Experiment 2).

Kelt Husbandry and Sampling

Initially, kelt were held at the laboratory without feeding in 2-m (1879-L) fibreglass tanks in ambient (2–4°C) freshwater at a stocking density of 0.02 kg/L. In December, all fish were individually identified by means of an intramuscular PIT tag (BioSonics, Inc., Seattle, WA) and they were treated with 0.10 mL (200 mg/mL) of erythromycin (P.V.U. Inc., Victoriaville, Que. G8P 1B1). Beginning in January the kelt were offered freshly thawed Atlantic silverside (*Menidia menidia*) supplemented with vitamins and trace minerals (Johnston et al. 1987). Initially, silversides were presented on a stick for insertion of the food directly into the mouth of kelt to stimulate their feeding behaviour (Johnston et al. 1990). Within a few sessions, some fish displayed interest in feeding on silversides tossed directly into the water. Eventually, all surviving kelt fed aggressively on silversides or chopped capelin (*Mallotus villosus*) offered 6 d/wk. The few kelt that refused to eat progressively lost weight until death which was usually coincident with rising water temperatures. At approximately monthly intervals the kelt were anaesthetized with 2-phenoxyethanol (232 ppm), weighed to the nearest gram, total fork length measured to the nearest centimetre, and 1-mL blood samples collected in heparinized syringes. Upon return to their tank, the kelt revived quickly and resumed feeding the next day. By September when the rate of food intake had greatly diminished, the body cavity of females was gently inflated with air for endoscopic inspection through the egg pore for positive signs of ovarian development, e.g. eggs > 3 mm. In ripening males the presence of milt was checked by gentle stroking of the abdomen. By late October, all females were examined weekly for signs of ripening ovaries e.g. softening of the body walls and enlargement/dropping of the egg pore. Soon after ovulation, the eggs in ovarian fluid were stripped from ovulated females into plastic bowls and the total egg number was derived from egg counts per 50 mL multiplied by the total volume of eggs collected from each female. After fertilization of eggs with the undiluted milt from at least two males, the eggs were rinsed with freshwater and water-hardened in the presence of a 0.1% iodine sterilizing solution. Subsequently, subsets of eggs from two or three kelt were combined for incubation at 7°C in Heath up-welling incubator trays. Kelt egg viabilities were determined by removing opaque eggs twice weekly and calculating the percent egg survival to eyed and to hatch and kelt larval survival to swim-up/first feeding and the fry stages where appropriate.

Radioimmunoassay Methods

Plasma vitellogenin (Vg) levels were determined according to So et al. (1985) after dilution of plasma samples 1:1000 and 1:10 000 with barbital buffer containing 0.07 mM bovine serum albumin and 0.25 mM thimerosol. In brief, the radioimmunoassay was performed by incubating the samples or Vg standards in serial dilution (100 μL in duplicate) with Vg antibody (200 μL at 1:250 000 dilution) and $[^{131}I]$Vg (200 μL containing about 20 000 cpm) at 4°C for 2 d. Finally, normal rabbit serum (100 μL at 1:80 dilution) and goat antirabbit-τ-globulin antibody (100 μL at 1:20 dilution were both added 24 h prior to centrifugation and counting of the precipitated bound radiolabelled Vg complex.

The steroid hormones estradiol-17β and testosterone were determined in undiluted plasma samples by radioimmunoassay methods using commercial reagents supplied by International Diagnostic Services Ltd. (IDS), Scarborough, Ont. After double ether extraction of the plasma, 100 μL (10%) of the ethanol-dissolved extract of plasma or steroid standard in ethanol (1–1000 pg) was pipetted in duplicate into 12 × 75 mm borosilicate glass tubes. Following evaporation of the ethanol under a stream of nitrogen, estradiol and testosterone were determined by bringing tubes to 0.4 mL with phosphate-buffered saline (PBS: 50 mM phosphate, 100 mM sodium chloride, 0.1% gelatin, pH 7.4) containing antiserum (100 μL) and approximately 10 000 cpm of iodinated estradiol or testosterone. Following a 1-h incubation at room temperature, 1 mL of ethylene glycol separating reagent was added to the incubation mixture to separate bound from free steroids. After another 25 min at room temperature, the tubes were centrifuged at 1650 × *g* for 15 min. Following centrifugation and aspiration of the supernatant, the tubes containing the precipitated bound steroid fraction were counted for 1 min in a gamma scintillation counter.

The 11-ketotestosterone assay was done with a previously described antibody (Truscott et al. 1986). Duplicate tubes were brought to 0.3 mL with PBS (0.03 M $NaH_2PO_4 \cdot H_2O$, 0.06 M Na_2HPO_4, 0.15 M NaCl, 0.1% gelatin, pH 7.0) containing antiserum at 1:70 000 final dilution and approximately 10 000 cpm of tritiated 11-ketotestosterone. Following overnight incubation at 4°C, 0.6 mL of dextran–charcoal suspension (1.25 g of charcoal and 0.25 g of dextran T-70 in 500 mL of PBS) was added and the incubation continued for 1 h at 4°C. After centrifugation of the tubes at 2200 × *g* for 15 min, the supernatant fluid containing the bound steroid fraction was decanted into a vial containing 10 mL of scintillation cocktail for beta counting.

Results and Discussion

Kelt Acclimation, Feeding, and Reconditioning

In Experiment 1, kelt were obtained in 1986 and subsequently held for two additional years (1987 and 1988) under laboratory conditions in ambient freshwater and a simulated natural photoperiod (Fig. 1). At relatively low water temperatures (approximately 4°C), very few kelt initially consumed silversides, even though the food was placed directly into their mouth. Subsequently, with more exposures to the silversides, kelt feeding interest resumed, particularly for silversides tossed directly onto the water surface; food consumption rose dramatically thereafter coincident with the increasing daylength and increased water temperatures in April–May (Fig. 1). Food intake began dropping after June and ceased in September when kelt had rematured as judged by increased plasma Vg and sex steroid hormone levels and ovarian development observed directly by endoscopic inspection. Most kelt survived during the winter (Fig. 1). An increasing number of mortalities were

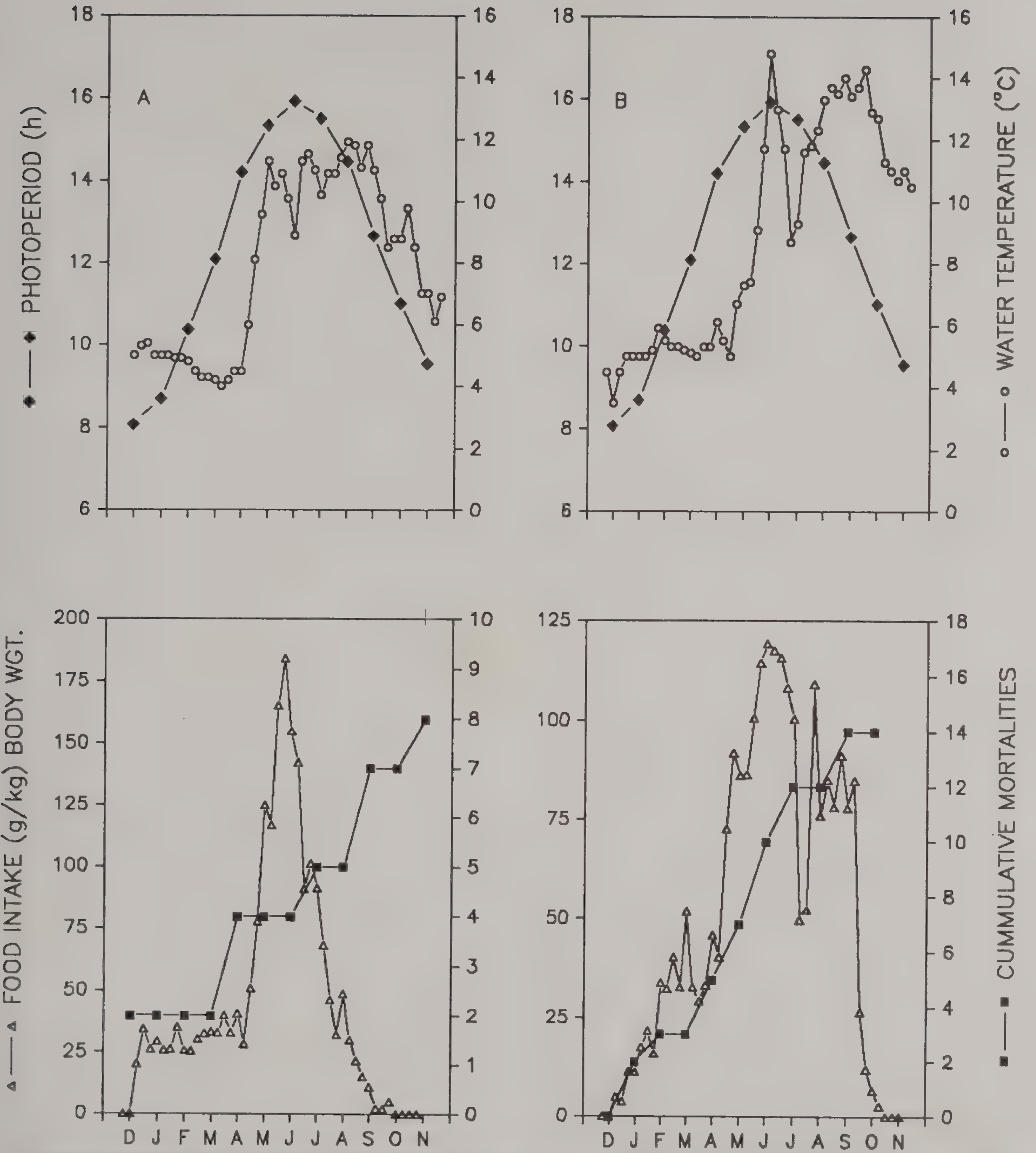

FIG. 1. Seasonal laboratory rearing conditions (photoperiod and water temperature) and records of average weekly food intake and cumulative monthly mortalities for Atlantic salmon kelt in Experiment 1. (A) 1987; (B) 1988.

observed during the spring and summer period of elevated water temperatures, primarily in fish with low condition factors (CF).

The monitoring of kelt CF in Experiment 1 indicated that progressive reconditioning occurred throughout the spring and early summer of 1987 (Fig. 2). After first rising significantly by April from the low winter CF values (<0.85), CF continued increasing into the summer and/or fall before they declined again following spawning. After spawning, these kelt, which were already adapted to laboratory culture conditions, again increased their consumption of silversides by April 1988 (Fig. 1), and a spring rise in CF (Fig. 2) was again observed. Kelt CF reached higher levels in the summer and fall of 1988 (second year of rearing in the laboratory) compared with the peak CF for 1987. In the second experiment beginning in 1988, while acclimating wild kelt to laboratory conditions, they were once again reluctant to feed at 4°C on the silverside diet (Fig. 3)

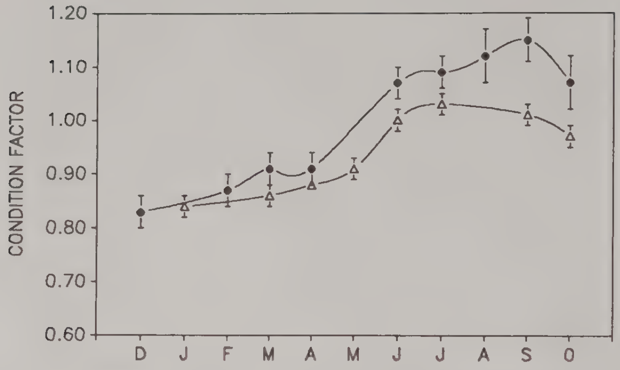

FIG. 2. Monthly CF (mean ± SEM) for Atlantic salmon kelt reared in the laboratory for two seasons, 1987 (open triangles) and 1988 (solid circles).

irrespective of maintaining kelt in freshwater (FW) or brackish seawater (SW). However, despite continued low water temperatures, food consumption of many kelt improved in February and March with additional exposure to silversides. Water temperatures increased in May and reached a peak in the summer of 13 and 15°C under SW and FW rearing conditions, respectively. Food consumption rose and remained elevated until late July when the feeding responses began declining towards basal values (Fig. 3). Mortality rates, at first low until May, rose in both groups of fish coincident with rising water temperatures (10 female mortalities in FW; 8 female mortalities in SW), and the deaths took place in Atlantic salmon of both low and high CF. Although no specific causes of kelt mortalities were identified, skin and gill smears from kelt have provided evidence of heavy ectoparasitic (*Trichodina truttae*) and rod-shaped bacterial infections in some fish (Khan 1991).

As before, progressive reconditioning of laboratory-reared Atlantic salmon kelt was demonstrated in Experiment 2 from the monthly monitoring of conditioning factors (Fig. 4). For the period from December to April, kelt CF remained depressed (<0.75), indicating the relatively poor condition of wild Atlantic salmon kelt following their prolonged period of starvation. Beginning in April, CF values significantly increased and they continued rising throughout the summer (mean CF $\leqslant 0.9$ in August) until the fall. It is interesting to note that beginning in January and continuing throughout the reconditioning period, higher CF were observed for second-time spawned, laboratory-reared kelt compared with the CF for wild kelt recently acquired from the field (Fig. 4).

The feeding of wild Atlantic salmon kelt in captivity is particularly difficult, since these fish are unaccustomed to laboratory holding conditions including high stocking conditions and the switch to unfamiliar commercial diets or homemade moist pellets. Factors responsible for the suppressed appetite observed in kelt are not known but could be specifically related to atrophy of their gastrointestinal tract and the poor body condition of kelt we have found following the long period of starvation experienced by postspawned Atlantic salmon. Extensive degeneration of the gastric and intestinal epithelium of migrating Atlantic salmon has been described by Gulland (1898). The extent of this degeneration may influence the feeding responses and rate of reconditioning of kelt. Other studies have demonstrated that degenerative changes occur in the digestive system of salmonids and other species during periods of starvation (Greene 1926; Robertson et al. 1961; Gas and Noailliac-Depeyre 1976; Boge et al. 1981). Hill (1978) reported that kelt, captured in the river in May or June during their seaward migration, could be readily fed on fresh diced fish and subsequently switched to a homemade prepared diet. Perhaps these kelt had already received feeding stimuli in the wild prior to their capture. Although the poor condition of Atlantic salmon kelt and their lack of interest in feeding in the winter have been previously reported, little is known about practical methods for improving the appetite of captive wild Atlantic salmon. We addressed this problem by feeding kelt the silverside diet which provides both visual and olfactory cues to stimulate the resumption of feeding (Johnston et al. 1987). Despite relatively low winter water temperatures (4–6°C) in January and February, some kelt would begin to feed on silversides situated at the surface of the water. Following a few silverside trials, kelt progressively and more aggressively increased their feeding responses on silversides tossed directly into the tank.

Increased water temperature in the spring appears to improve the appetite of starved Atlantic salmon, since Hill (1978) showed that kelt held at 15°C resumed feeding earlier and reconditioned more quickly compared with fish held at 6°C. Johnston et al. (1987) also found that 12°C water temperatures better stimulate the feeding response compared with low ambient water temperatures. Gray et al. (1987) reconditioned kelt on a moist pellet diet and noted that, despite the fact that most kelt did not eat at low temperatures until mid-May, they obtained high survival rates over the first (93%) and the second year (83%) in captivity. Dumas et al. (1991) reported that, early in the year at 8–10°C, kelt began feeding well on a silage-based moist pellet diet, and food intake improved with increasing water temperatures. The feeding responses and the rate of reconditioning of kelt may also be influenced by the circulating level of thyroid hormones, L-thyroxine (T4) and 3,5,3-triiodo-L-thyronine (T3), since spring increases in water temperatures and photoperiod are matched with a rise in plasma T4 and T3 for kelt held in freshwater (Eales et al. 1991). Whereas elevations in thyroid hormones occurred in feeding kelt with increased appetite and rate of reconditioning, kelt that failed to feed and recondition in the spring had low T3 and T4 values. T3 levels also decreased to very low values during peak estradiol secretion and heightened vitellogenesis, a time when food consumption in kelt dramatically declined.

Kelt Rematuration

Following a year of reproductive inactivity during 1986, 88% (21 of 24) of the surviving female kelt in Experiment 1 rematured in 1987, producing eggs for a second time (Table 1). After spawning in the fall of 1987, seven of these postspawned females died prior to the time for detection of onset of gonadal rematuration in August. Of 12 surviving, twice-spawned females, 10 kelt were rematuring for a third time in 1988 without additional missing of annual reproductive cycles (Table 1). Eight of 12 rematuring females survived to spawn by November 1988; egg fecundity (Table 1) was not significantly different between females spawning for a second (1987) and third time (1988). Although one female (F23) failed to remature over the course of two consecutive reproductive seasons (1986 and 1987), this fish rematured and spawned for the second time in 1988. Two other females (F47 and F51) spawned a second time in 1987 but failed to remature for the third time in 1988. Of eight male kelt, six (75%) rematured and were spermiating by the fall of 1987. Five male kelt died during the 1988 rearing

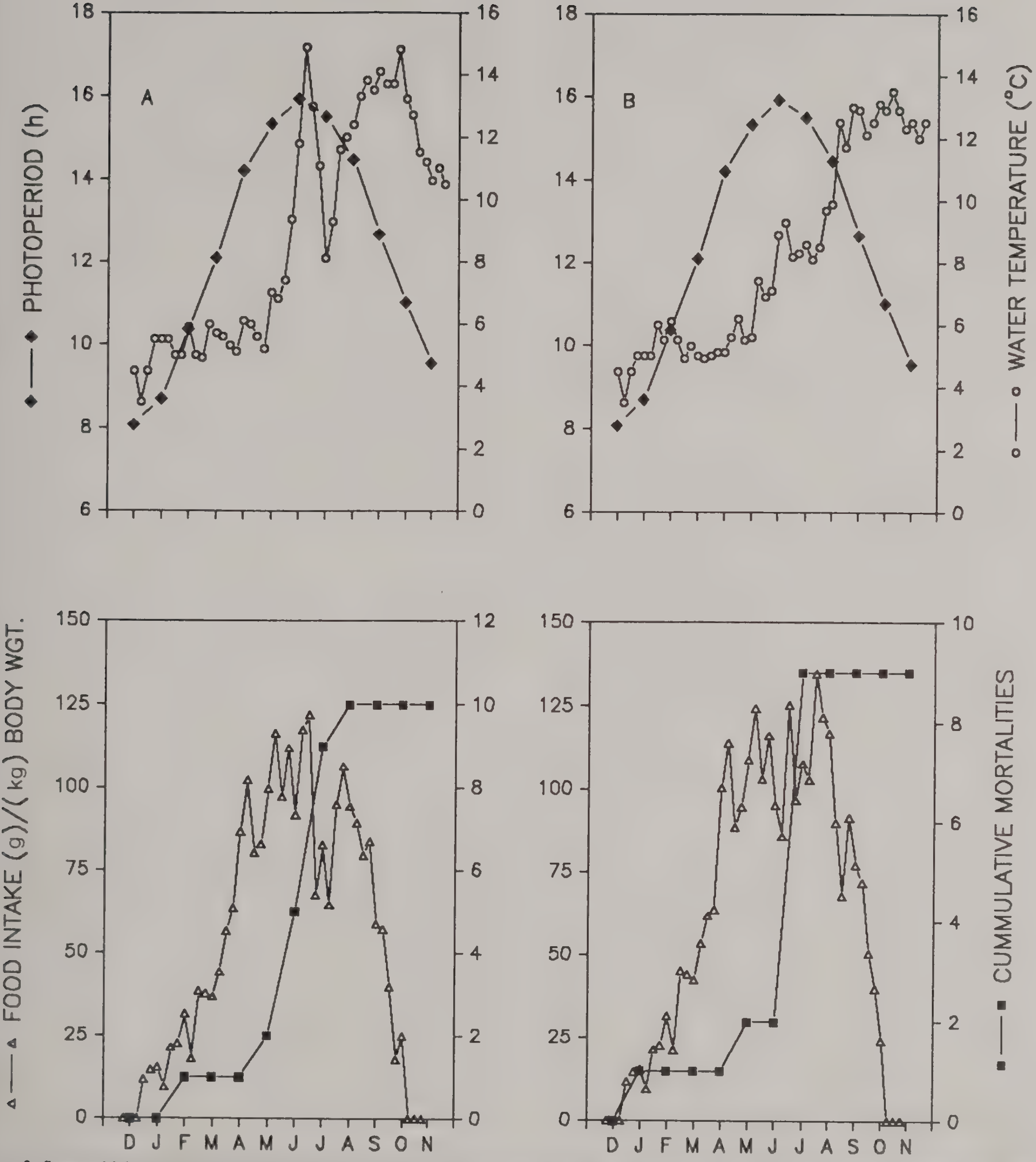

FIG. 3. Seasonal laboratory rearing conditions for Experiment 2 (photoperiod and water temperature) and records of weekly average food intake and cumulative monthly mortalities for Atlantic salmon kelt reared in (A) FW and (B) SW.

season; by November 1988, the one surviving male rematured and spermiated for a third time.

A low rate of rematuration occurred for female kelt (2 of 18 = 11%) in Experiment 2 with just one female from each group (FW and SW) demonstrating reproductive activity. Of the limited number of male kelt included in this study, just 4 of 11 surviving males rematured (three in FW, 43%; one in SW, 25%), suggesting that the rate of rematuration may be higher for reconditioned males compared with females.

Consecutive repeat spawning of wild Atlantic salmon, up to five or six times, was reported by Ducharme (1969). Our present work, clearly indicating that reconditioned kelt have the capacity to reproduce many times, confirms and extends previous studies of Atlantic salmon kelt showing that these fish

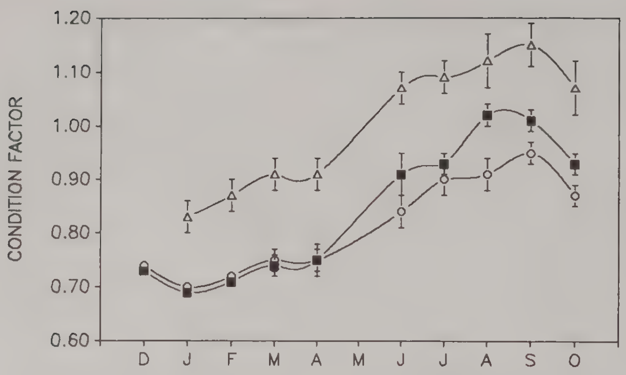

FIG. 4. Monthly CF (mean ± SEM) for Atlantic salmon kelt reared in FW (open circles) or SW (solid squares) in Experiment 2. CF data (open triangles) for FW-reared kelt (Experiment 1, 1988) repeated from Fig. 2 for comparison.

from the wild may be reconditioned and mature under captive conditions (Johnston et al. 1987, 1990; Gauthier et al. 1989).

Pepper and Parsons (1987), in examining the aquaculture potential of kelt, reported that the incidence of rematuration for kelt was less than 2%. In agreement with their observations, we demonstrated that most of our reconditioned Atlantic salmon kelt failed to remature in a consecutive fashion after their first year in captivity. Indeed, our data suggest that a very low percentage of reconditioned grilse kelt in either FW or SW will immediately undergo repeat maturation during their initial year in captivity. Notably, after skipping a single seasonal reproductive cycle, most females rematured consecutively for a second and a third time. Similar results were obtained with reconditioned kelt in seawater by Gray et al. (1987) who reported a low rate of rematuration, particularly in female kelt (18 and 41% in females and males, respectively), after their first year in captivity. After the second year, the rate of rematuration increased to 83% in females and 96% in the males. Johnston et al. (1987, 1990) reported a variable rate of rematuration for female kelt held in freshwater ranging from 0 to 56%. Their best results (highest rate of rematuration) were obtained when females were held under natural photoperiod conditions in either ambient or elevated water temperatures (12°C).

The reports by Hill (1978) and Gauthier et al. (1989) provide evidence in favour of high rates of rematuration for female Atlantic salmon kelt without missing annual reproductive cycles. Recently, Dumas et al. (1991) obtained similar results in France with indigenous Atlantic salmon stocks. It appears, therefore, that the propensity for Atlantic salmon kelt to remature either sporadically or repeatedly may be stock dependent. It is interesting to speculate upon the seasonal timing for the kelt rematuration decision and the reasons why Atlantic salmon may not undergo consecutive rematuration following the completion of their initial reproductive cycle. Clearly, during the nonfeeding period from January to March, CF are higher (≥0.80 versus ≤0.70) for kelt reconditioned in the laboratory during the previous year (spawners of the year) compared with reproductively inactive, long-term starved kelt recently acquired from the wild (Fig. 2). In other words, if the decision to remature occurs in the spring of the year as proposed for precocious maturation of Atlantic salmon parr (Rowe and Thorpe 1990), then CF are higher in reconditioned kelt the year after their reproductive inactivity. These data support the concept that body energy stores are related to control of sexual rematuration in Atlantic salmon and perhaps certain levels of body fat or protein are required for triggering reproductive development as suggested for control of maturation of cultured Atlantic salmon (Thorpe et al. 1990). Of course, other factors could play an equally important role in induction or suppression of reproductive development of Atlantic salmon such as genetic background, stress levels, feeding levels, and environmental conditions such as light and temperature (Skarphedinsson et al. 1985; Saunders et al. 1983, 1987).

Kelt Vg and Hormone Profiles

Plasma Vg and the sex steroid hormones remained relatively low in nonrematuring female kelt throughout the 1987 rearing season including the time of year normally expected for gonadal recrudescence in Atlantic salmon from August to October (data not shown). In contrast, plasma Vg and the sex steroids increased markedly in rematuring females during the period of gonadal development in both their second (1987) and third (1988) reproductive cycles (Fig. 5). By June in rematuring

TABLE 1. Year of rematuration and egg fecundities for wild female kelt which skipped a year of reproduction after spawning for the first time in 1985 (Experiment 1).

Identification	Weight (kg)	Times rematured[a]		Fecundity (eggs/kg)	
		1987	1988	1987	1988
F5	1.7	2	3	2490	—[b]
F7	3.4	2	3	943	1384
F14	1.8	2	3	1705	1368
F24	2.5	2	3	1151	1456
F25	1.6	2	3	2209	1571
F38	4.0	2	3	645	1206
F39	1.5	2	3	1221	—[b]
F40	2.3	2	3	2607	—[b]
F46	2.2	2	3	1351	915
F48	2.8	2	3	1481	2035
F23	2.2	n	2	—	1283
F47	1.8	2	n	1824	—
F51	2.1	2	n	1738	—

[a] n indicates no maturation; skipped a year of reproduction.
[b] Died during rematuration before spawning.

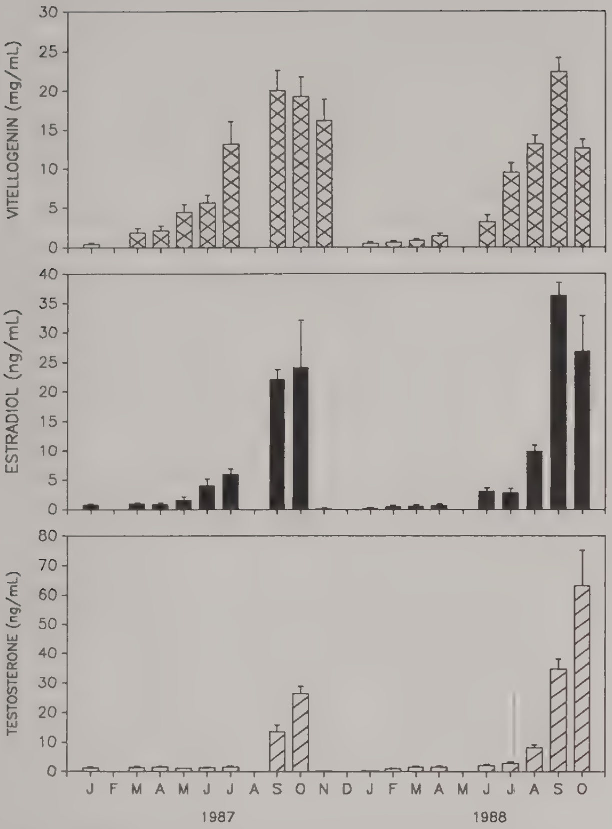

FIG. 5. Monthly determinations (mean ± SEM) of Vg, estradiol, and testosterone in laboratory-reared maturing female kelt in 1987 and 1988. Values obtained for nonreproductive females too low for presentation.

females, plasma estradiol was rising and increased to maximum values by September–October near the time for spawning (Fig. 5). Plasma testosterone was also detectable during the reproductive period in female kelt, but substantial increases in testosterone were delayed until September and October (Fig. 5). The earliest indications of sexual rematuration of females were signalled by the appearance of Vg in the plasma first noted in March. Plasma Vg levels continued rising to a peak in September and October. By the time of spawning in fully ripe females, the levels of both estradiol and testosterone markedly declined in contrast with plasma Vg which remained elevated.

The studies of So et al. (1985) indicated that plasma levels of Vg correlate with gonadal development in female landlocked Atlantic salmon. It was recently suggested by So et al. (1989) that both Vg and the sex steroid hormones may serve as markers for early detection of rematuration of Atlantic salmon kelt. Whereas these substances remained low during the reproductive season in nonmaturing kelt, clear increases in Vg, estrogen, and testosterone were observed in female kelt rematuring for their second and third reproductive cycles. It appears that profiles for Vg and the sex steroid hormones in rematuring female kelt compare favourably with the seasonal changes in Vg in landlocked Atlantic salmon (So et al. 1985) and for the sex steroids in Atlantic salmon females spawning for the first time (Crim et al. 1986).

In rematuring males, low but detectable plasma testosterone levels were observed until August or September when distinct increases in plasma testosterone were correlated with the period of rapid gonadal development in August or September (Fig. 6). By September, plasma 11-ketotestosterone levels were also greatly increased and exceeded the plasma concentration of testosterone during the period of spermiation in ripe male kelt. The progressive changes in sex steroid hormones in male kelt closely match the seasonal sex steroid pattern described for captive male Atlantic salmon by Hunt et al. (1982) who demonstrated that the highest seasonal levels of serum androgenic hormones occurred in males in conjunction with the onset of milt expression. Interestingly, these investigators also detected 11-ketotestosterone in males in the early months of the year well before onset of gonadal development. Indeed, kelt plasma hormone profiles also provided evidence of low levels of sex steroids during the early months of the year, perhaps yielding an early indication of maturation in males.

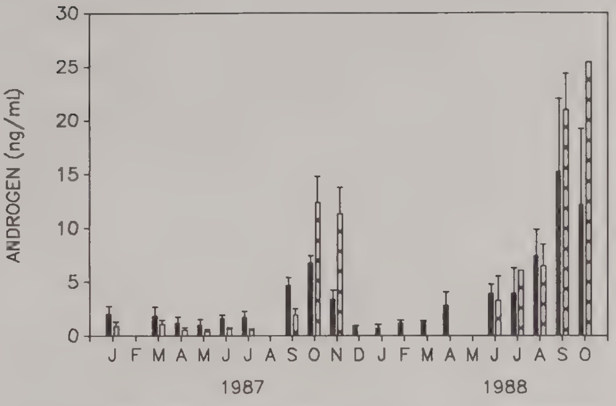

FIG. 6. Monthly determinations (mean ± SEM) of plasma testosterone (solid bars) and 11-ketotestosterone (hatched bars) in laboratory-reared maturing male kelt in 1987 and 1988.

Kelt Egg Quality

Despite the high rates of egg fertilization (mean% = 96.5 ± 0.0001; 93.7 ± 0.0013) and survival of eggs to hatch (mean% = 92.7 ± 0.0023; 90.4 ± 0.0022) observed in 1987 and 1988, respectively, the majority of batches of kelt larvae died suddenly prior to swim-up, approximately 5 wk after hatch, suggesting that kelt egg quality is very poor. Nevertheless, some viable egg batches were produced by three females in 1987 (none in 1988) as evidenced by the production of swim-up larvae (mean survival to swim-up = 63%) and 20% survival for these young Atlantic salmon fry ranging in weight from 2 to 5 g.

Little information is currently available regarding the quality of kelt reproductive products, although Hill (1978) described heavy egg losses during the first 60 d of incubation of fertilized kelt eggs. In contrast with the progeny of Atlantic salmon kelt, better survival for Atlantic salmon eggs collected from first-time spawners has been reported by both Hill (1978) and Dumas et al. (1991). Our present results, indicating poor kelt egg quality, differ from previously reported results only in that we consistently observed low mortality of kelt eggs to hatch and including the early larval rearing periods. By the time the kelt Atlantic salmon larvae reached the stage of first feeding, however, heavy larval losses or complete loss of egg lots were experienced. In 1987, we considered kelt egg quality to be relatively good, since eggs from some females survived producing viable swim-up larvae which were successfully reared to the fry stage before termination of the experiment. In 1987, the kelt broodstock was fed chopped capelin in addition to the vitamin-supplemented silversides, suggesting the presence of an unknown deficiency in the silverside diet. Although we have not identified a particular dietary deficiency, kelt feeding on silversides (composition = 15.6% protein, 7.2% fat, 3.1% ash and 71.9% water) at peak capacity ($18\ g\cdot kg^{-1}\cdot d^{-1}$) would receive a relatively low level of protein (approximately 3 g/d) compared with the protein intake (8 g/d) for Atlantic salmon fed a standard 40% protein commercial diet.

In subsequent experiments, we have observed much improved survival rates for larvae produced by captive Atlantic salmon kelt fed throughout their lives on a standard commercial salmon diet (unpublished results). The importance of the broodstock diet was clearly demonstrated in the previous work of Gray et al. (1987) and Dumas et al. (1991) who reported survival rates of 74% to the alevin stage and >38% to the yolk resorption stage, respectively, for the F1 kelt progeny. Although there seems to be an obvious connection between the chopped fish diet of our kelt broodstock and poor egg quality, a number of other factors might also account for the high mortality rates of kelt progeny in our experiments including frequent handling of broodstock, excessive summer water temperatures, and high water temperatures in the fall near the time for spawning.

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Straying Patterns of Coho Salmon (*Oncorhynchus kisutch*) Stocks from Southeast Vancouver Island, British Columbia

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Labelle, M. 1992. Straying patterns of coho salmon (*Oncorhynchus kisutch*) stocks from southeast Vancouver Island, British Columbia. Can. J. Fish. Aquat. Sci. 49: 1843–1855.

Fourteen coho salmon (*Oncorhynchus kisutch*) stocks of wild and hatchery origin were tagged from 1985 to 1988 in nine streams within a 150-km coastal section of Vancouver Island. Tag escapements to natal streams were estimated from fence counts, stream surveys, and mark–recapture operations. On average, adult (age 3+) strays accounted for ~4.7% of escapements, but for >40% of the adult escapements in some cases. Adult straying rate, averaged across all years and stocks, was <2%. Straying rates tended to be lower for hatchery fish and highest for stocks subjected to certain enhancement practices. Adult strays escaped to streams 2–159 km from their home stream (average 15.7 km); over 50% escaped to streams <7 km from their stream of release. Straying rates of jacks (age 2+) in a given year and that of their adult siblings during the following year were not related. Genetic makeup, age-at-return, run timing, and exposure to nonnatal water sources during the rearing stage did influence homing. Changes in natural straying patterns should be suspected where enhancement measures include flow controls, selective breeding, and exposure of fry to various water sources. Straying levels and stray contributions should be considered when estimating survival and exploitation rates

De 1985 à 1988, on a étiqueté des saumons cohos (*Oncorhynchus kisutch*) sauvages et d'élevage prélevés de 14 stocks de neuf cours d'eau répartis sur 150 km du littoral de l'île Vancouver. L'échappée de saumons étiquetés aux cours d'eau d'origine a été établie à partir de dénombrements effectués à des barrières de comptage des poissons, de relevés des cours d'eau et d'expériences de marquage et de recapture. En moyenne, les adultes (3+ ans) n'appartenant pas au stock local constituaient environ 4,7 % de l'échappée, mais ce pourcentage pouvait atteindre plus de 40 % dans certains cas. La moyenne du taux d'abandon du stock d'origine dans le cas des adultes, établie pour cette période et tous les stocks, était inférieure à 2 %. Ce taux avait tendance à être moins élevé chez les saumons d'élevage; il était le plus élevé chez les stocks faisant l'objet de mesures de mise en valeur. Les adultes n'appartenant pas au stock local concerné ont remonté des cours d'eau situés de 2 à 159 km de leur cours d'eau d'origine (distance moyenne : 15,7 km); ainsi, plus de 50 % ont remonté des cours d'eau situés à moins de 7 km du cours d'eau où ils ont été mis en liberté. Dans le cas des jeunes mâles précoces (2+ ans), le taux d'abandon du stock d'origine au cours d'une année donnée n'était pas lié à celui des adultes du même stock au cours de l'année suivante. Le patrimoine génétique, l'âge au moment de la remonte, le moment de la remonte et l'exposition à d'autres sources d'eau lors de l'élevage ont influé sur le retour vers les eaux natales. On doit s'attendre à des modifications du régime naturel d'abandon du stock d'origine lorsque les mesures de mise en valeur comprennent la régulation du débit, l'élevage sélectif et l'exposition des alevins à diverses sources d'eau. On devrait tenir compte du taux d'abandon du stock d'origine et de la contribution des saumons étrangers dans l'estimation des taux de survie et d'exploitation.

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One of the distinguishing features of the Pacific salmon life history is their ability to return back to their natal stream to spawn and die after a period of ocean residency. Various theories have been proposed to account for this "homing" ability (Smith 1985), which is generally assumed to be fairly accurate (see Brannon 1982). Larkin (1978) noted that there was no evidence to support the view that a measurable part of the natural mortality at sea could be accounted for by salmon becoming "lost". However, there are numerous well-documented cases of salmon "straying" to streams other than their natal one, which is essentially the process by which new habitats get colonized.

Information on the amount of straying for a given population is useful for stock assessment purposes, since it serves to clarify the relation between stock and recruitment, and improve estimates of total survival rates. Knowledge of the factors which affects straying can also be used to improve enhancement practices and assess the effects of environmental impacts. Various studies have already provided some evidence that straying is substantial for some populations (Shapavalov and Taft 1954) and that straying can be influenced by genetic factors and enhancement practices (Bams 1976), spawner age, and season (Quinn and Fresh 1984). However, a review of the existing information on straying led Quinn and Fresh to state that "reliable estimates of the proportion of salmon that home are virtually non-existent".

An investigation was conducted during 1984–89 in southern British Columbia to determine the similarity among coho salmon (*Oncorhynchus kisutch*) populations. One objective of the study was to quantify straying rates within a local assemblage of wild and hatchery salmon stocks. A secondary objective was to identify the population attributes, hatchery practices, and environmental variables that influence homing of coho salmon. Since all stocks were subject to coded-wire tagging and escapement enumeration, information was available on stream location, flow patterns, smolt size and time of ocean entry, run timing, adult size and age-at-return, year of return, exposure to enhancement activities, hatchery rearing practices, genetic differences, exploitation patterns, and survival rates. As a result, it was possible to provide estimates of straying among these stocks based on tag recovery patterns and assess the effects of the above factors on homing.

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Materials and Methods

Nine streams on the east side of Vancouver Island were selected for this study on the basis of accessibility, geographical location, coho abundance levels, hydrological regimes, availability of logistic facilities, and exposure to enhancement activities. The streams were the Quinsam River, Black Creek, Puntledge River, Trent River, Rosewall Creek, Big Qualicum River, Little Qualicum River, French Creek, and the Millstone River (Fig. 1). Details on the biological and physical features of the streams and populations monitored during this study have been reported by Hancock and Marshall (1985), Reinhardt and MacKinnon (1979), Fraser et al. (1983), Hamilton (1978), Hurst and Blackman (1988), Lukyn et al. (1985), Bocking et al. (1988), and Labelle (1990a).

The various ''types'' of coho found in these streams were classified as follows: ''wild'' stocks consisting of coho that spawn and rear under natural conditions, ''production'' stocks consisting of coho reared entirely under hatchery conditions, ''colonization'' stocks consisting of coho reared under hatchery conditions for a few months and released as fry (<10 g) into the stream to complete their growth before smolting, and ''enhanced'' stocks consisting of a mixture of wild coho and unmarked hatchery coho from colonization releases. Some of the streams selected were occupied by several identifiable types of coho, so a total of 14 stocks were available for comparative purposes. For the present study, coho from different streams were considered to be genetically distinct, except when one stream population consisted of the progeny of spawners from another stream where brood stock was collected. In such cases, the progeny was considered to be genetically similar to the sibling population in the parent stream.

To generate reliable information on the life history traits of each stock, juvenile tagging and escapement enumeration were conducted in all streams. Coded-wire tags (CWT)(Jefferts et al. 1963; Jewell and Hager 1972) were used for this purpose, since this technology has been shown to be cost-effective for large-scale juvenile tagging programs and because Pacific salmon fisheries and escapements are systematically sampled for CWT

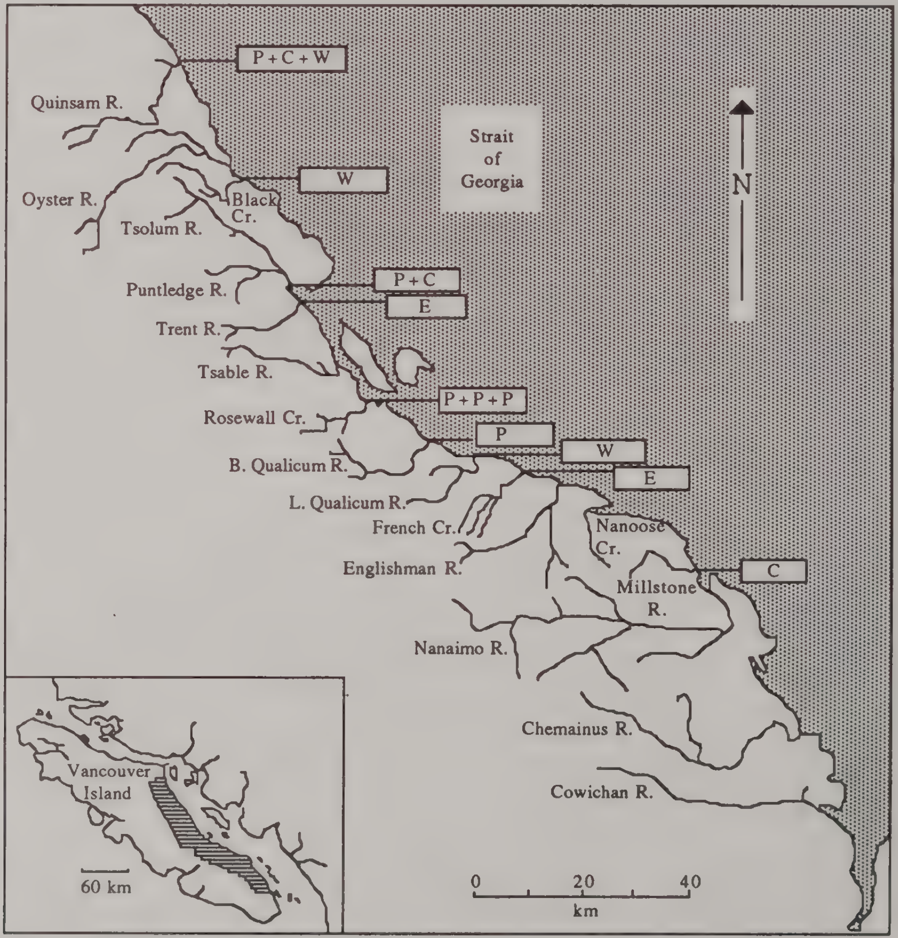

FIG. 1. Geographical location of study site. The abbreviations used to represent the stocks in each stream are as follows: P, production; C, colonization; E, enhanced; W, wild.

recovery. Juveniles from each stock were tagged and released during the springs of 1985, 1986, and 1987. Escapement enumeration and tag recovery were conducted at the release sites and in the adjacent systems during the 1985–88 period. Juvenile tagging, escapement estimation, and tag recovery operations differed substantially among streams, so a brief description of the procedures used at each location is presented here.

Juvenile Tagging

Large public hatcheries are located on the Quinsam, Puntlege, and Big Qualicum rivers. English et al. (1991) gave a detailed account of the hatchery production and sampling programs conducted at these locations by hatchery personnel. Coho were reared in the holding ponds and rearing channels located in areas adjacent to each river. Several weeks prior to release, a fraction of the fry population in each pond or channel was fin clipped and tagged. Total fry abundance was then determined by mark–recapture methods. Dead fry recovered subsequently were deducted from the mark–recapture estimates to determine the remaining fry populations. English et al. noted that these ''book estimates'' can be positively biased, since undetected losses due to predation and decomposition were not included. Samples were subsequently taken several weeks after tagging to estimate tag loss, which was theoretically a sufficient time to account for all tag loss (Blankenship 1990). Juveniles released from each facility as smolts after an 18-mo rearing period were considered to be individual production stocks. Some of the fry and smolts released from these facilities during 1984 and 1985 (1983 brood) were also given a characteristic mark (left ventral fin excised) to conduct detailed assessment of the 1986 fishery contributions.

Some of the coho reared at the Puntledge River and Quinsam River facilities were released in the headwaters of these systems as fry during their first summer after being fin clipped, tagged, and sampled for tag loss. These two groups were classified as colonization stocks. At Puntledge River, the number of tagged smolts that survived and migrated to sea each year was based on the number of fry released, and the average fry-to-smolt survival estimated from previous surveys conducted by hatchery personnel. At Quinsam River, smolt traps installed at the main fence upstream from the hatchery were used to determine the number of tagged smolts from colonization releases that migrated to sea each year. The traps were also used to capture and enumerate wild (unmarked) smolts leaving the river. Wild smolts exhibiting no visible injuries were fin clipped, tagged, and released within a 48-h period after samples were checked for tag loss. This latter group was classified as a wild stock.

At Little Qualicum River, a large spawning channel is used primarily for the production of chum salmon (*Oncorhynchus keta*). However, some adult coho also enter the channel each year and spawn. Since the progeny rear in the channel under seminatural conditions, this group was considered to be a wild stock. A sampling device was installed at the exit of the channel each year to determine the total coho smolt output. Smolts exhibiting no visible injuries were fin clipped, tagged, and released within a 48-h period after samples were checked for tag loss.

At the Millstone River, hatchery-reared coho were released as fry during their first summer in the headwaters. The brood stock used for this program was collected each fall in this river and transported to the nearby Nanaimo River hatchery for rearing. A few days prior to release, the fry were fin clipped, tagged, and checked for tag loss after a 48-h retention period. Smolt traps were used each year to determine the number of tagged smolts leaving the system. Details of the trapping and tagging operations were reported by Hurst and Blackman (1988), who also noted that the hatchery-reared coho accounted for the vast majority of the smolt output of this system. The tagged group was classified as a colonization stock.

At Black Creek, Trent River, and French Creek, downstream smolt traps (Conlin and Tutty 1979) were installed to tag smolts throughout the migration period each year. Fin clipping and tagging were conducted in areas adjacent to the trap, with the same procedures as used in neighboring hatcheries. Tags were applied exclusively to juvenile coho exhibiting smolting characteristics (Wedemeyer et al. 1980), lacking visible injuries, and exceeding 7 cm in fork length. All smolts were released within 48 h from the period of capture after samples were checked for tag loss. Tagged coho from Black Creek were classified as a wild stock, since the natural production was not supplemented with the addition of hatchery fry. At French Creek and Trent River, brood stock were collected each fall for enhancement purposes. The fry were reared separately in small artisanal hatcheries located nearby and released unmarked during the first summer in the respective streams. Given the lack of a characteristic fin clip, smolts from colonization releases were not clearly distinguishable from wild smolts during tagging, but Labelle (1990a) estimated that hatchery-reared coho accounted for >10% of the smolts from French Creek and 40–60% of the smolts from Trent River. The tagged groups from these two streams were classified as enhanced stocks.

During 1984 and 1985, brood stock were collected at Black Creek, Trent River, and the Little Qualicum River, and the progenies from each group were reared separately at the Rosewall Creek hatchery. A few weeks prior to release, the fry were fin clipped, tagged, and examined for tag loss after a 48-h retention period. All smolts were released simultaneously each year at times which corresponded as much as possible to the peak migration periods of their natural counterparts. These coho were classified as production stocks of different broods.

Escapement Estimation and CWT Recovery

At the Quinsam, Puntledge, Little Qualicum, and Big Qualicum rivers, permanent fences diverted most of the returning coho to holding channels. At Rosewall Creek, an electric fence (Burrows 1957) diverted most returning coho into concrete raceways. Most of the coho held at these sites were sexed, aged (jack/adult), measured, examined for missing fins, and processed for sales, brood stock acquisition, and tag recovery. Estimates of the number of adults and jacks that spawned and died in the stream were obtained through interviews with managers of each facility, who have surveys conducted for such purposes. These figures were combined with those from the hatchery to determine total escapement, sampling rates, and overall tagged proportions by age category for each system.

At Black Creek, Trent River, and French Creek, collapsible fences designed by engineers of the Salmon Enhancement Program (SEP) were used for escapement enumeration. Fish intercepted at the fence were identified, aged (jack/adult), examined for missing fins, and usually marked on the operculum prior to release. Stream surveys were then conducted periodically to determine trends in marked proportions throughout the season, estimate the proportion of each escapement lacking specific fins (fin-clipped proportions), and recover tagged heads from carcasses. Mark–recapture estimates of jack and adult escapements were generated separately based on fence counts and stream survey data. Details of the sampling regimes and the mark–recapture estimation procedures used were reported by Labelle (1990a).

To obtain additional information on movement patterns of coho in the Trent River, 43 adult coho were captured with seine nets in pools from the middle reaches, approximately 9 d after their entry into this river. Serially numbered tags were applied to the coho, which were released within 60 s from the time of capture (see Bocking et al. 1988 for details). To determine if hatchery strays (fin clipped) and nonhatchery coho spawned together, efforts were made to capture spawners paired together near redds, or in the process of spawning. All fish captured were examined, sexed, measured, and released within 60 s. No attempt was made to dig out the redds to determine the extent of egg fertilization.

At the Millstone River, trained technicians from the Nanaimo River hatchery conducted foot surveys and sampling activities during the period of peak spawner abundance to determine escapement levels, acquire brood stock, and recover tagged heads from carcasses. Estimates of escapements by age category and the corresponding fin-clipped proportions were obtained form hatchery records. Additional surveys were also conducted periodically by Department of Fisheries and Oceans (DFO) personnel each fall to determine escapements to other streams in the same region. The rivers surveyed included the Quatsese, Oyster, Little Oyster, Tsable, and Chase. The creeks surveyed included Granite Bay, Coal, Waterloo, and Chef. Estimates of escapements, fin-clipped proportions, and sample sizes were obtained directly from the surveyors. In the rare cases where sample sizes or escapement estimates were not available, the number of tagged coho recovered (“select recoveries”) (Kuhn et al. 1988) was used as the total number of tagged coho of a given code that escaped to the stream.

Statistical Treatments and Analytical Methods

Heads collected each season from escapement samples were sent to DFO's Head Recovery Laboratory for identification or processed at the hatcheries by experienced tag readers. Data on tag identities and sampling rates are stored in the Mark–Recapture Database (MRP) along with pertinent statistics on juvenile attributes, tagging procedures, and rearing conditions. This database is maintained by personnel from DFO at the Pacific Biological Station in Nanaimo, British Columbia. All statistics used in the following analysis were extracted from this database according to the procedures described by Kuhn (1988).

Not all coho lacking an adipose fin in a sample are successfully dissected and positively associated with a given code. These “nontags” (Kuhn et al. 1988) include coho that were not dissected (no data; ND), those that were dissected but the tag was lost (lost pin; LP), and those that lacked a tag (no pin; NP). Specific methods were used to account for these nontags based on the recovery source and the availability of ancillary information. The methods used were similar to those proposed by Kuhn et al. Sample statistics were first corrected for sampling deficiencies and the reported emigration of marked coho from some streams. Nontags were then associated with certain codes to estimate the expected number of tags of a given code in a sample (adjusted recoveries). These were then expanded according to the sampling rate to estimate the expected number of tags in the escapement (estimated recoveries). It should be emphasized that these estimates are generated for each age class (jacks/adults) separately. The major steps in this estimation process are described here, but further details can be obtained from Labelle (1990b).

For escapement samples from Rosewall Creek and the Quinsam, Puntledge, and big Qualicum rivers (hatchery systems), the frequencies in the various nontag categories were first corrected to account for the misidentification of 1% of the unclipped coho, which was hypothesized to occur during the sorting stage at the last three locations (DFO unpublished internal report). The adjusted recoveries in escapement samples were then estimated as follows:

$$(1)\quad T_{\mathrm{corr},c} = T_{\mathrm{obs},c} \cdot \left(1 + \frac{\mathrm{LP}}{K}\right) \cdot \left(1 + \frac{\text{tag loss}_c}{1 - \text{tag loss}_c}\right)$$

$$(2)\quad K_{\mathrm{corr}} = \sum_{c} T_{\mathrm{corr},c}$$

$$(3)\quad T_{\mathrm{adj},c} = T_{\mathrm{corr},c} \cdot \left(1 + \frac{\mathrm{ND}}{K_{\mathrm{corr}}}\right)$$

where $T_{\mathrm{obs},c}$ = number of observed recoveries of code c in the sample, $T_{\mathrm{adj},c}$ = estimate of adjusted recoveries of code c in the sample, tag loss_c = estimate of tag loss associated with code c, and K = number of identified tags in the sample (Σ observed recoveries).

The number of estimated recoveries for each code ($T_{\mathrm{tot},c}$) in the escapement (esc) was obtained after accounting for the sampling rate and the nondetection of adipose-clipped coho (8%) which was assumed to occur during the sorting stage (DFO unpublished internal report):

$$(4)\quad T_{\mathrm{tot},c} = \frac{T_{\mathrm{adj},c}}{0.92} \cdot \frac{\mathrm{esc}}{\mathrm{sample}}.$$

Sampling error was considered to be negligible in all other streams monitored. The number of NP associated with all tagged fish of stray origin (nearly always from hatchery systems) in an escapement sample was estimated by multiplying the contributing stray tag codes by their corresponding tag loss estimates. This estimate was then deducted from the total number of NP in the escapement sample to obtain the number of NP associated with tag codes of nonstray origin escaping back to their stream of release. The ratio NP/(K + NP) based on nonstray statistics was considered as the tag loss estimate for nonstray tagged groups in the sample, since these were not held long enough before release to account for all tag loss. This tag loss estimate was assumed to apply to all codes of nonstray origin in the sample, since roughly equal numbers of coho were usually tagged with each code at release. This tag loss estimate was also used to determine (by Eq. 1–3) the number of adjusted recoveries for the associated tag codes, in hatchery systems and other streams where strays were detected. For streams where no strays were detected, there was simply no need to allocate nontags based on code-specific estimates of tag loss, so nontags were allocated in proportion to the contribution of each code in the escapement samples:

$$(5)\quad T_{\mathrm{adj},c} = T_{\mathrm{obs},c} \cdot \left(1 + \frac{\mathrm{NP} + \mathrm{LP} + \mathrm{ND}}{K}\right)$$

$$(6)\quad T_{\mathrm{tot},c} = \frac{T_{\mathrm{adj},c} \cdot \mathrm{esc}}{\mathrm{sample}}.$$

Estimates obtained by Eq. 4 and 6 represent the number of tagged coho of a given stock, release year, age class, and code that escaped to a given stream and year. The tag escapement for a given group (combination of stock, release year, and age class) to one stream and year was obtained by pooling the estimated recoveries across all tag codes associated with the group. The total tag escapement for each group was estimated by further pooling across all streams:

$$\text{tag esc}_g = \sum_{st} \sum_{c} T_{\text{tot,st,}c} \tag{7}$$

where tag esc_g = total tag escapement for a given group (g) and $T_{\text{tot,st,}c}$ = estimated recoveries with code (c) in a stream (st).

For each group, homing proportions were obtained from the ratio of the tag escapement in the stream of release to the total tag escapement. Straying rates were defined as 1.0 minus the homing proportion, or the fraction of the group that escaped to streams other than the stream of release.

The contribution of strays from hatchery systems to each escapement was obtained by expanding the estimated recoveries of each stray tag code to account for the associated untagged smolts released. For each of these codes, the corresponding tagged proportions at release ($p(\text{tagged})_{\text{rel}}$) were estimated from

$$p\,(\text{tagged})_{\text{rel},c} = \frac{\text{tag rel}_c}{\text{total rel}_c} \tag{8}$$

where tag rel_c = total number of smolts released that were tagged with code c, adjusted for mortalities and tag loss prior to release, and total rel_c = estimate of total smolt output represented by code c, adjusted for mortalities prior to release.

The total number of coho associated with each code (c) that strayed to a given stream (stray_c) and the contribution of strays associated with all foreign tag codes ($C = \Sigma c$) to each escapement (expressed as the proportion: $p(\text{stray})_{\text{esc}}$) were estimated as

$$\text{stray}_c = \frac{T_{\text{tot,}c}}{p(\text{tagged})_{\text{rel,}c}} \tag{9}$$

$$p(\text{stray})_{\text{esc}} = \frac{\sum_{C} \text{stray}_c}{\text{esc}}. \tag{10}$$

Note that the above estimation procedure does not account for the contribution of strays from unassociated releases: groups of fry released from a hatchery, or transplanted to neighboring streams, that do not contain tagged individuals and that cannot be readily associated with another tagged group. However, such groups represented a relatively minor portion of the total production of public hatcheries during the 1984–88 period (MRP database records).

To determine the contribution of strays from nonhatchery systems, the above estimation procedure was slightly modified. Trapping and tagging operations conducted by means of smolt traps did not provide accurate estimates of total smolt output, so tagged proportions were based on escapement statistics. Escapements to nonhatchery systems were first adjusted to account for stray contributions to estimate escapements of coho returning to their stream of release. Estimated recoveries for these nonstray groups were expanded by a factor of 1.19 to account for the hypothesized differential mortality of tagged and untagged smolts at release that is induced by trapping and tagging operations (see Blankenship and Hanratty 1990). This expanded figure divided by the corrected escapement was used as the tagged proportion estimate for each group returning to their stream of release. The contribution of strays from these groups to other streams was then estimated from Eq. 9 and 10.

Effects of Various Factors on Homing

A linear logistic model (Fienberg 1980) was used to describe the structural relationship between the tag recovery patterns and various factors monitored during this study. The objective was to predict the probability of homing for different types of coho characterized by particular combinations of population attributes and environmental factors. The logistic model describing this relationship is

$$E\left(\frac{r_1}{r_2}\right) = \frac{\exp^{(b_0+b_1x_1+b_2x_2+\ldots)}}{1+\exp^{(b_0+b_1x_1+b_2x_2+\ldots)}} \tag{11}$$

where r_1 = estimated number of tagged coho of a group that escaped to its home stream, r_2 = estimated number of tagged coho of a group that escaped to all streams, b_n = parameters to be estimated, and x_n = population attribute (x_1 = year of return, x_2 = smolt size, etc.).

The relative frequencies in each category (r_1, r_2) was considered to be the response variable of interest. The population attributes tested for their effects were x_1 = calendar year of escapement (1–4, representing 1985–88), x_2 = genotype (parental stock) of the group released (the eight categories used were Quinsam (1), Black Cr. (2), Puntledge (3), Tent (4), B. Qualicum (5), L. Qualicum (6), French Cr. (7), and Millstone (8); wild, colonization, and production coho residing in the same sream were considered to be members of the same category; Rosewall Creek releases from different brood stocks were categorized according to the parent stock used (2, 4, and 6), x_3 = duration of the hatchery rearing period in months (the estimates used were wild = 0, enhanced ≈ 3, colonization ≈ 6, production ≈ 18), x_4 = smolt weight at release (in grams), x_5 = median date of smolt migration (calendar date), x_6 = release location, expressed as the latitudinal distance (in kilometres) from Nanaimo, x_7 = starting date of the upstream spawner migration, x_8 = median date of the upstream spawner migration, or run timing (calendar date), x_9 = age of the spawners, in terms of ocean residency period (jacks = 1, adults = 2), x_{10} = exposure to nonnatal (foreign) water sources during the rearing stage (Y/N), x_{11} = total number of escapement recoveries, and x_{12} = existence of mechanical flow control in the stream of release (Y/N).

The stepwise logistic regression program (LR) of the BMDP statistical package (Dixon et al. 1988) was used to fit the logistic model to the data. Given the relatively low number of distinct observations (stock/year combinations) in relation to the large number of factors that could be incorporated into the model, it was necessary to impose limits on the number and type of interactions tested simultaneously. Main factors related to each other ($r^2 > 0.50$) were identified by means of correlation matrices, and only one factor from each correlated pair was allowed into the model. From the remaining set, only the main factors and their first-order interactions were tested. The first-order interactions tested were those hypothesized to have an influence on the trait of interest through some plausible mechanism.

The objective of the model fitting process was to determine which source contributed most to an improvement in fit, which was accomplished by examining the reduction in "deviance" obtained by the inclusion of additional factors in the model (as suggested by Green and Macdonald 1987). For the present study, additional factors were incorporated into the model until the reduction in deviance associated with the additional factors was ≤5% of the largest reduction in deviance obtained during previous inclusions. As a result, factors which had a relatively small contribution to the improvement in fit were not incorporated in the model, even if their contribution was ''statistically'' significant. By omitting factors with marginal contributions, the resulting model was more parsimonious, yet could still be used for ''predictive'' purposes, since it accounted for the effects of the main factors.

Once a suitable model was identified, a sensitivity analysis was performed by conducting goodness-of-fit tests with different versions of the model characterized by the presence or absence of each factor. Further insight into the direction of changes in homing resulting from variation in the levels of each factor was obtained by incorporating the regression coefficient estimates generated by the BMDP program into the logistic model and then predicting homing proportions associated with particular combinations of parameter values arbitrarily selected within a realistic range.

Results

Stray Contributions and Straying Rates

Information on tag identities revealed that the majority of the tagged adults recovered in the Trent River during 1986 were of Puntledge River origin (Tables 1 and 2) and that adult strays contributed about 44.8% of the total escapement to this river (Table 3). However, such high stray contributions were not typical, since in the majority of cases, strays contributed less than 1% of the escapements of adults and jacks. Stray contributions, averaged across all streams, accounted for ~3% of the jack escapements and ~4.7% of the adult escapements. Such high average contribution estimates were mainly induced by the relatively large contribution of strays to the Trent River, which accounted for more than half of the coho escaping to this river in 1987. Adults and jacks tended to stray more to the Puntledge River than to other large hatchery systems such as the Big Qualicum and Quinsam rivers. Strays in the Puntledge River consisted mainly of coho reared at the Puntledge River hatchery and released in the headwaters of the adjacent river (Tsolum River) for colonization purposes.

The accuracy of hatchery stray contributions based on expansion of tag recoveries was assessed based on the proportions of fin-clipped coho observed in the 1986 escapements to nonhatchery systems. The proportion of adults lacking a left ventral fin in the 1986 escapement at Trent River was estimated to be about 40.1%. Given that about 82% of the smolts released from the Puntledge River hatchery were fin clipped, the number of fin-clipped adults in the Trent River should be expanded by 1.22 to account for the unclipped smolts released. Based on this expanded figure, it was estimated that adults from the Puntledge River hatchery accounted for ~48.9% of the 1986 Tent River escapement, which is similar to the estimate based on expansion of tag recoveries (44.8%). At French Creek, fin-clipped adults accounted for less than 0.8% of the escapement, which is comparable with the figure obtained by expansion of tag recoveries (0%). Such results indicate that the contribution of strays can be reliably estimated by expansion of tag recovery data.

Straying rates among adults ranged from 0 to 54.1%, but for most groups, a high proportion of the tagged coho escaped to their home stream (Tables 1 and 2). Among adults, straying was lowest for production releases (Table 4). On average, adults from colonization releases strayed slightly more than those from production releases. Adults from wild populations strayed slightly more than the two former types, and adults from enhanced populations strayed more than those of wild populations. Adults from experimental releases conducted at the Rosewall Creek hatchery exhibited unusually high levels of straying during 1987, but straying in 1988 was only slightly greater than average. Chi-square tests of the stray:nonstray frequencies revealed significant differences among stock types each year, but no clear pattern was consistent across all years. On average, adults exhibited more straying in 1987 than in other years. On an overall basis, adult straying averaged 1.5%, but if the experimental releases from Rosewall Creek were omitted, the average was 1.0%. Adult strays escaped to streams located 2–159 km from their home stream, but the average straying distance was 15.7 km, and over 50% of the strays escaped to streams located less than 7 km from their home stream.

Straying rates among jacks ranged from 0 to 50%. As for adults, production and colonization releases from large hatchery systems exhibited very little straying. Those from experimental releases at Rosewall, and colonization releases at the Millstone River, strayed the most. On average, straying appeared to be greatest during 1988 and 1986. No significant correlation was detected between the level of straying among jacks in a given year and their adult siblings during the following year. On an overall basis, jack straying averaged 0.43%, and if the experimental releases from Rosewall Creek were omitted, the average was 0.38%, which is substantially less than was observed for adults. Jack strays escaped to streams located 2–91 km from their home stream, but the average straying distance was 26.3 km, and over 50% of the strays escaped to streams located less than 7 km from their home stream.

Some of the Trent River strays were recovered in the Puntledge River, which serves as the water source for the artisanal hatchery used to rear the colonization fry. In addition, some adults of Trent River brood stock released from the Rosewall Creek facility were recovered at the Trent River. However, in the majority of cases where adults and jacks strayed from their location of release, they were recovered in streams which were neither their ancestral stream nor the stream where they reared for a portion of their presmolt life.

Effects of Various Factors on Homing

Not all variables tested contributed to a substantial improvement in fit (Table 5). The duration of the hatchery rearing period per se (x_3) was not required in any of the models, indicating that this factor had relatively little influence on homing. All remaining variables were useful in predicting homing proportions, with some of them being incorporated into the model only as interaction factors. Model 9 was judged to be an appropriate model for describing homing proportions. The predicted values were highly correlated with the observations, and no pattern was detected in the residuals. More complex models incorporated mainly interactions of factors already included in model 9 and failed to provide a substantial improvement in fit as judged by the trajectory of the reduction in deviance. This indicates that information on return year, rearing conditions, genetic makeup, run timing, and flow control was sufficient for predicting homing proportions. Factors related to the juvenile migration (smolt migration date, smolt size, release location) had a relatively minor influence.

Results of the sensitivity analysis indicated that the genetic × exposure interaction factor was a major determinant of homing proportions (Table 6) and suggest that certain stocks are more susceptible than others to straying when exposed to for-

TABLE 1. Estimates of the number of tagged jacks from each population that escaped to selected streams (recovery locations) from 1985 to 1988. Numbers accompanied by a ">" sign are minimum estimates of tag escapements obtained from select samples. % homing indicates the proportion of the total tag escapement that escaped to their stream of release.

Year	Release site and stock[a]	Quatsese 300	Granite 143	B. Quins. 141	Oyster 120	Black 118	Little 101	Puntl. 99	Trent 93	Tsable 80	Coal 75	Waterloo 74	Rosewall 73	Chef 71	B. Qual. 59	L. Qual. 50	French 41	Millst. 8	Chase 0	Sum	% homing
		Recovery locations (km from Chase, Nanaimo)																			
1985	Quins. (p)		>4	1687											2					1693	99.6
	Quins. (c)		>2	305																307	99.3
	Black Cr.					88														88	100.0
	Puntl. (p)		>2					3109	1											3112	99.9
	Puntl. (c)							15												15	100.0
	Trent R.																			0	—
	B. Qual.														203					203	100.0
	L. Qual.								1							56				57	98.2
1986	Quins. (p)			1128					2											1130	99.8
	Quins. (c)			213																213	100.0
	Quins. (w)			1																1	100.0
	Black Cr.					268														268	100.0
	Puntl. (p)							88												88	100.0
	Puntl. (c)							3												3	100.0
	Trent R.								6						1					7	85.7
	Rosew. (b.c)												66							66	100.0
	Rosew. (t.r)												30							30	100.0
	Rosew. (l.q)												27							27	100.0
	B. Qual.														80					80	100.0
	L. Qual.															52				52	100.0
	French Cr.																7			7	100.0
	Millstone							1										7	6*	14	50.0
1987	Quins. (p)			1018																1018	100.0
	Quins. (w)			19																19	100.0
	Black Cr.					417									1					418	99.8
	Puntl. (p)							218												218	100.0
	Puntl. (c)							5												5	100.0
	Trent R.							2	39						1					42	92.9
	Rosew. (b.c)												25							25	100.0
	Rosew. (t.r)												13		5					18	72.2
	Rosew. (l.q)												64		3					67	95.5
	B. Qual.														494					494	100.0
	L. Qual.														1	261				262	99.6
	French Cr.												1		9	1	184			195	94.4
	Millstone																	12		12	100.0
1988	Quins. (p)			3072											2					3074	99.9
	Black Cr.					266									6					272	97.8
	Puntl. (p)							207												207	100.0
	Puntl. (c)							17												17	100.0
	Trent R.							2	144											146	98.6
	B. Qual.														284					284	100.0
	French Cr.														4		168			172	97.7
	Millstone																	>2	>1	3	66.7

[a] Letters in parentheses are stock type or origin if different from release site: p, production; c, colonization; w, wild; bc, Black Creek; tr, Trent River; lq, Little Qualicum River. See text for further explanation.

eign water sources during the rearing stages. Run timing and flow controls were also necessary for predicting homing, and their influence upon homing varied from year to year (because of the interaction). Run timing was also found to influence the homing of various age classes and stocks to a different extent.

Predictions of homing proportions made with the logistic models indicated that homing was negatively affected by the exposure to nonnatal water sources during rearing stages. For all stocks, a reduction in the age-at-return (adult to jack) caused the predicted homing rate to increase by a few percentage points, which supports the previous observation that jacks strayed less than adults.

Predicted homing proportions also improved in the presence of flow control, but the magnitude of the improvement differed from stock to stock. Accordingly, delays in run timing caused the predicted homing proportions to decrease. A 1-wk delay caused a 6 percentage point reduction in homing proportions (88–82%) for adults returning to the Trent River in 1986, but only a 0.5 percentage point reduction in the predicted homing proportions of Trent River jacks returning that year. The difference is attributed to the influence of the age × run time interaction and suggests that jacks are less likely to stray than adults due to delays in fall freshets. The magnitude of the reduction in the predicted homing proportions of Trent River adults was found to differ slightly in other years for comparable delays in run timing. This was expected, since the year × run time interaction was a factor in the model and a determinant of homing. Of particular interest is the finding that delays in run timing led to a slight increase in the predicted homing proportions of stocks returning to streams characterized by flow controls. During this study, the starting and median date of upstream migration in large systems with flow controls (Quinsam, Puntledge, Big Qualicum, and Little Qualicum rivers) generally occurred several weeks earlier than in the other streams. These results suggest that early runs are more likely to stray than later runs.

The influence of genetic factors on homing proportions can be assessed by contrasting the three groups released from the Rosewall Creek hatchery. None of the brood stocks used exhibited consistently greater or lesser adult homing proportions during 1987 and 1988. By contrast, adults returning to the Trent River exhibited consistently lesser homing than those returning to Black Creek and Little Qualicum River. Thus, in the absence

TABLE 2. Estimates of the number of tagged adults from each population that escaped to selected streams (recovery locations) from 1985 to 1988. Numbers accompanied by a "<" sign are minimum estimates of tag escapements obtained from select samples. % homing indicates the proportion of the total tag escapement that escaped to their stream of release.

		Recovery locations (km from Chase, Nanaimo)																			
Year	Release site and stock[a]	Quatsese 300	Granite 143	B. Quins. 141	Oyster 120	Black 118	Little 101	Puntl. 99	Trent 93	Tsable 80	Coal 75	Waterloo 74	Rosewall 73	Chef 71	B. Qual. 59	L. Qual. 50	French 41	Millst. 8	Chase 0	Sum	% homing
1985	Quins. (p)			7472																7472	100.0
	Quins. (c)			614																614	100.0
	Puntl. (p)							3079	19											3098	99.4
	Puntl. (c)							144												144	100.0
	B. Qual.														414					414	100.0
1986	Quins. (p)			5388				2	4											5394	99.9
	Quins. (c)			1301				4												1305	99.7
	Black Cr.			2		824	>2													828	99.5
	Puntl. (p)							2152	128					>3	7					2290	94.0
	Puntl. (c)							164												164	100.0
	Trent R.							6	64						2					72	88.9
	B. Qual.														307					307	100.0
	L. Qual.															99				99	100.0
1987	Quins. (p)			1715										>2						1717	99.0
	Quins. (c)			996																996	100.0
	Quins. (w)	>10		118																128	92.2
	Black Cr.				>19	558														577	96.7
	Puntl. (p)							175	16											191	91.6
	Puntl. (c)							113												113	100.0
	Trent R.							6	158						9					173	91.3
	Rosew. (b.c)							2	16				58	>2	21					99	58.6
	Rosew. (t.r)								64				79	>2	25	2				172	45.9
	Rosew. (l.q)										>1		34		4					39	87.2
	B. Qual.														165					165	100.0
	L. Qual. R.														4	245				249	98.4
	French Cr.														7		51			58	87.9
	Millstone														2			27		29	93.1
1988	Quins. (p)			892																892	100.0
	Quins. (w)			95																95	100.0
	Black Cr.					1541														1541	100.0
	Puntl. (p)							382	7				1							390	97.9
	Puntl. (c)							104												104	100.0
	Trent R.							45	384				1							430	89.3
	Rosew (b.c)											>1	139		4	5				149	93.3
	Rosew. (t.r)												152		2					154	98.7
	Rosew. (l.q)							1					176		6					183	96.2
	B. Qual.														276	3				279	98.9
	L. Qual.															628				628	100.0
	French Cr.															3	623			626	99.5
	Millstone																	172		172	100.0

[a]Letters in parentheses are stock type or origin if different from release site: p, production; c, colonization; w, wild; bc, Black Creek; tr, Trent River; lq, Little Qualicum River. See text for further explanation.

TABLE 3. Estimates of the contribution of strays to each escapement based on expansion of CWT recoveries. The contributions are expressed as a fraction of the total escapement × 100.

	Jacks				Adults			
Stream	1985	1986	1987	1988	1985	1986	1987	1988
Quins. R.	0	0.03	0	0	0.03	0.02	0	0
Black Cr.	0	0	0	0	0	0	0	0
Puntl. R.	0	2.6	0.3	0.1	0	7.6	8.6	9.1
Trent R.	29.2	69.1	0	0	25.3	44.8	50.2	4.6
Rosew. Cr.	—	0	1.5	0	—	0	0	1.6
B. Qual. R.	1.0	0	0.3	0.3	0	0.9	1.6	0.2
L. Qual. R.	0	0	0.4	0	0	0	0.2	5.9
French Cr.	0	0	0	0	0	0	0	0
Millstone. R.	—	0	0	0	—	0	0	0

of experimental releases from the Rosewall Creek hatchery, it might have been hypothesized that genetic factors were responsible for the differences in homing observed among stocks from the three streams. As suggested by the structure of model 9, genetic factors affect homing, but this effect is influenced by hatchery rearing practices (genetic × exposure). Adult homing proportions for 1987 were predicted for these three stocks under the assumption of no differences in fry rearing regimes and run timing. The predicted homing of Black Creek adults decreased by less than 1 percentage point, but that of Trent River adults increased to levels comparable with the two other stocks. This suggests that the lower homing proportion of adults escaping to the Trent River occurred because some juveniles were exposed to nonnatal water sources during rearing.

Hatchery rearing effects can also be assessed by comparing the homing proportions of coho released from the same loca-

TABLE 4. Proportion (× 100) of total tag escapement of each population that strayed to other streams and average straying distances (km). Q.R., Quinsam River; B.C., Black Creek; P.R., Puntledge River; T.R., Trent River; R.C., Rosewall Creek; B.Q., Big Qualicum River; L.Q., Little Qualicum River; F.C., French Creek; M.R., Millstone River. Groups: p, production; c, colonization; w, wild; e, enhanced. Abbreviations for Rosewall Creek production releases refer to the broodstocks used: bc, Black Creek; tr, Trent River; lq, Little Qualicum River. BY figures represent differences in straying between the two corresponding age classes (jacks and adults).

	Q.R. (p)	Q.R. (c)	Q.R. (w)	B.C. (w)	P.R. (p)	P.R. (c)	T.R. (e)	R.C. (bc)	R.C. (tr)	R.C. (lq)	B.Q. (p)	L.Q. (w)	F.C. (e)	M.R. (c)	Mean
Proportion (× 100) of total tag escapement															
Adults															
1985	0	0			0.6	0					0				0.1
1986	0.1	0.3		0.5	6	0	11.1				0	0			2.2
1987	0.1	0	7.8	3.3	8.4	0	8.7	41.4	54.1	12.8	0	1.6	12.1	6.9	11.2
1988	0		0	0	2.1	0	10.7	6.7	1.3	3.8	1.1	0	0.5	0	2.0
Mean	0.0	0.1	3.9	1.3	4.3	0.0	10.2	24.1	27.7	8.3	0.3	0.5	6.3	3.5	6.5
Jacks															
1985	0.4	0.7		0	0.1	0					0	1.8			0.4
1986	0.2	0	0	0	0	0	14.3	0	0	0	0	0	0	50	4.6
1987	0		0	0.2	0	0	7.1	0	27.8	4.5	0	0.4	5.6	0	3.5
1988	0.1			2.2	0	0	1.4				0		2.3	33.3	4.9
Mean	0.2	0.4	0.0	0.6	0.0	0.0	7.6	0.0	13.9	2.3	0.0	0.7	2.6	27.8	
BY															
1985 rel.	0.3	0.4		−0.5	−5.9	0					0	1.8			−0.6
1986 rel.	0.1	0	−7.8	−3.3	−8.4	0	5.6	−41.4	−54.1	−12.8	0	−1.6	−12.1	5.6	−9.3
1987 rel.	0		0	0.2	−2.1	0	−3.6	−6.7	26.5	0.7	−1.1	0.4	5.1	0	1.5
Mean	0.1	0.2	−3.9	−1.2	−5.5	0.0	1.0	−24.1	−13.8	−6.0	−0.4	0.2	−3.5	2.8	
Average straying distances (km)															
Adults															
1985					6										6.0
1986	46	42		20	8		13								25.8
1987	70		159	2	6		23	16	18	12		9	18	51	34.9
1988					9		6	17	14	16	9		9		11.4
Mean	58	42	159	11	7.25		14	16.5	16	14	9	9	13.5	51	
Jacks															
1985	29	2			31							43			26.3
1986	48						34							19.9	34.0
1987				59			15		14	14		4	19		20.8
1988	82			59			6						19	8	34.8
Mean	53.0	2.0		59.0	31.0		18.3		14.0	14.0		23.5	19.0	14.0	

TABLE 5. Composition of various logistic models describing the homing proportion of all stocks during the 1985–88 period. The main factor and interactions included in each model are listed with the corresponding degrees of freedom (df) and deviance (G). The factors are year (x_1), genetic (x_2), hatchery rearing period (x_3), smolt weight (x_4), smolt migration date (x_5), stream location (x_6), run timing (x_8), spawner age (x_9), exposure to foreign water sources (x_{10}), total number of escapement recoveries (x_{11}), and flow control (x_{12}).

Model	Main factors	Interactions	df	G
0	Constant (k)	—	81	2202
1	k, x_{10}	—	80	1317
2	k, x_{10}, x_{11}	—	79	1212
3	k, x_{10}, x_{11}	$x_8 \times x_9$	78	1077
4	k, x_{10}, x_{11}	$x_8 \times x_9, x_1 \times x_{12}$	75	832
5	k, x_{10}, x_{11}	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8$	68	503
6	k, x_{10}, x_{11}, x_9	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8$	67	463
7	k, x_9	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8, x_2 \times x_{10}$	62	392
8	k, x_9	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8, x_2 \times x_{10}, x_1 \times x_8$	59	369
9	k, x_9, x_1	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8, x_2 \times x_{10}, x_1 \times x_8$	56	321
10	k, x_9, x_1	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8, x_2 \times x_{10}, x_1 \times x_8, x_6 \times x_8$	55	302
11	k, x_9, x_1	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8, x_2 \times x_{10}, x_1 \times x_8, x_6 \times x_8, x_9 \times x_{12}$	54	290
12	k, x_9, x_1	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8, x_2 \times x_{10}, x_1 \times x_8, x_6 \times x_8, x_9 \times x_{12}, x_9 \times x_{10}$	53	274
13	k, x_9, x_1	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8, x_2 \times x_{10}, x_1 \times x_8, x_6 \times x_8, x_9 \times x_{12}, x_9 \times x_{10}, x_{10} \times x_{11}$	52	263
14	k, x_9, x_1	$x_8 \times x_9, x_1 \times x_{12}, x_2 \times x_8, x_2 \times x_{10}, x_1 \times x_8, x_6 \times x_8, x_9 \times x_{12}, x_9 \times x_{10}, x_{10} \times x_{11}, x_5 \times x_6$	51	252

TABLE 6. Relative influence of various factors on homing proportions predicted from model 9. Goodness-of-fit tests were performed in the presence and absence of each factor. The factor removed precedes the minus sign. The difference in chi-square obtained after the removal of the factor is given under the Difference column. Parenthetical df are df of factor omitted.

Model	Factor omitted	Log-likelihood	Chi-square	Difference	df	P
Main + interaction (M + I)	None	−1992.80	320.911	—	56	0.0000
$M + I - x_1$	Year	−2016.96	369.231	48.32	(3)	0.0000
$M + I - x_9$	Age	−2001.77	338.851	17.94	(1)	0.0000
$M + I - x_2 \times x_{10}$	Genetic × exposure	−2088.74	512.801	191.89	(7)	0.0000
$M + I - x_1 \times x_8$	Year × run time	−2017.56	370.441	49.53	(3)	0.0000
$M + I - x_2 \times x_8$	Genetic × run time	−2015.38	366.071	45.16	(7)	0.0000
$M + I - x_1 \times x_{12}$	Year × flow control	−2006.19	347.701	26.79	(3)	0.0000
$M + I - x_8 \times x_9$	Run time × age	−2002.98	341.271	20.36	(1)	0.0000

tion, but exposed to different hatchery rearing periods. For the Quinsam and Puntledge rivers, homing of adults from colonization releases tended to be slightly greater than that of the production release (Table 2), but chi-square tests of the stray:nonstray frequencies failed to detect significant differences in most cases. In addition, no significant differences were detected between the stray:nonstray frequencies of adults of wild and production origin returning to the Quinsam River in 1987 and 1988. This supports the finding that hatchery rearing period was not a major determinant of homing. However, the same test revealed significant differences between the three Rosewall Creek groups and their counterparts returning to Black Creek and Trent and Little Qualicum rivers in all cases for both years (all P's < 0.01). Adult homing proportions of the three Rosewall Creek groups were nearly always lower than those of their jack counterparts. Since the duration of hatchery rearing was not found to be a major determinant of homing, the lower homing of the Rosewall Creek groups was most likely induced by their later run times and differences in rearing conditions.

Additional Observations on Coho Behavior

Most of the coho with serially numbered tags were subsequently recovered in areas adjacent to the location of tagging. However, one tagged adult was recovered 2 wk after release in the headwaters of the Tsolum River, ~15 km from the tagging location. Such facts indicate that adult coho can move upriver for several kilometres before leaving the stream.

Field observations also revealed that several coho left the Trent River during the first flood each year, and coho with opercular marks were found in neighboring streams after the floods. Most of these emigrants went to the nearby Puntledge River hatchery, but their stream of origin could not be determined with certainty because the tags were not identified. After accounting for the sampling regime at the hatchery, the number of marked adult coho that emigrated from the Trent River during the 1986, 1987, and 1988 seasons was estimated to be 181, 8, and 12 adults, respectively. This represented 16.8, 4.1, and 3.3% of the adults counted at the Trent River fence and provides some information on the magnitude of the emigration that can occur after the initial upstream migration. Based on the time of recovery and the occurrence of floods on the Trent River, it was assumed that the marked coho left the stream on November 18, 1986, November 11, 1987, and November 1, 1988. None of the marked coho released at the other fences were ever recovered in adjacent streams, even though floods occurred each year in those systems.

Significant differences in the relative abundance of fin-clipped adults were detected between the upper and lower sections of the river during the 1986 season ($\chi^2 = 5.5, P = 0.02$). Samples from the upper sections (beyond the China Creek junction) contained proportionally fewer fin-clipped adults than samples from lower sections, indicating that hatchery strays tended to remain in the lower reaches of the river. There was also a reduction in the relative abundance of fin-clipped adults in samples taken before and after the flood, which is to be expected from the departure of fin-clipped hatchery coho returning to the Puntledge River hatchery. However, this reduction was not statistically significant ($P = 0.175$) because only a small proportion of the fin-clipped coho left the stream after the floods.

Examination of the coho captured on the spawning grounds revealed that hatchery strays were paired with fin-clipped and unclipped coho. Assuming that some unclipped coho were of nonhatchery origin, and that such pairing occasionally lead to successful spawning, then some hybridization of hatchery and nonhatchery fish occurred in the Trent River.

Discussion

Based on recovery patterns of coded-wire tagged coho, Quinn and Tolson (1986) estimated that stray contributions to the Quinsam River jack and adult escapements during 1980 were 0.08 and 0.33%, respectively, and were nonexistent for the 1981–83 period. Average stray contributions to the 1978–83 Big Qualicum River jack and adult escapements were 0.07 and 0.06%, respectively, and no strays were found in escapement samples from the Puntledge River (Quinn and Tolson 1986). Only one of the tagged strays in escapement samples had been released from the Big Qualicum River hatchery, and none of these had been released from the other two hatcheries (Quinn and Tolson 1986). Such figures suggest that straying among these hatchery populations was negligible during the 1978–83 period.

The results of the present study are in agreement with those of Quinn and Tolson (1986) and with circumstantial evidence from field surveys and inferences from genetic studies. None of the tagged jacks or adults sampled from the Quinsam River escapements were of Puntledge River and Big Qualicum River origin (Tables 1 and 2). None of the tagged jacks in the Puntledge River escapement samples were from the Big Qualicum or Quinsam River, and only two of the tagged adults were of Quinsam River origin. The number of tagged jacks from the Quinsam and Puntledge rivers found in the Big Qualicum River escapement samples were less than two per year, and only during 1986 were tagged adults from the Puntledge River detected in the Big Qualicum River escapement samples. Estimates of straying among hatcheries for the 1986–88 period were slightly greater than reported by Quinn and Tolson, but this is undoubtedly due in part to the corresponding increase in tagging effort and escapement sampling rates. If this is true, additional tagging of juveniles in adjacent streams should also induce higher stray contributions than reported by Quinn and Tolson. Accordingly, the stray contributions to the hatchery escapements of jacks, averaged across the 1985–88 period (from Table 3), ranged from 0.01% for the Quinsam River to 0.75% for the Puntledge River. Stray contributions to adult escapements ranged from 0.01% for the Quinsam River to 6.3% for the Puntledge River.

Shapavalov and Taft (1954) reported that 14.9% of the adult coho returning to Waddell Creek in California strayed to Scott Creek, a coastal stream located 8 km north of it. During their study, some of the hatchery-reared coho released at Scott Creek also strayed to Waddell Creek. Jacobs (1988) reported the results of an escapement survey of Oregon coastal streams aimed at determining straying levels of hatchery coho subjected to accelerated growth and released as preyearlings after a brief exposure to salt water. Based on the tag recovery patterns, Jacobs estimated that adult coho strayed considerably (range 1–30% of escapement) and strayed predominantly to basins located within a 40-km radius from their point of release. Hatchery-reared coho transported offshore prior to release were twice as likely to stray as those released from onshore facilities. The results of these investigations support the findings of the present study that straying rates of natural and enhanced coho stocks can be considerable and that rearing practices can affect straying patterns.

Bams (1976) conducted a series of experimental releases of pink salmon (*Oncorhynchus gorbuscha*) fry in the Tsolum River, using the progeny of a hybrid stock (Tsolum River × Kakweiken River cross). Differences in treatment effects between the various groups released were minimal. Based on the return rates observed, Bams concluded that adults from the transplanted hybrid stock homed less than those of the other stock. Note that Bams estimated homing rates based on the relative number of recoveries in the stream of release and not on the basis of tag recovery patterns across several streams. Further examination of the figures reported by Bams shows that the only marked adults recovered in adjacent streams were of Tsolum River stock. Bams might have therefore reached the opposite conclusion if his data had been analyzed in accordance with the procedure used in the present study. The results of his study could be viewed as support for the notion that homing is affected by the genetic factors. An alternative interpretation is that homing accuracy is affected by human manipulaton. The results of the present study indicate that the interaction of both of these factors can influence homing, and efforts should be made to account for their separate effects in further studies. Evidence of an hereditary component to the homing behavior of chinook salmon (*Oncorhynchus tshawytscha*) was given by McIsaac and Quinn (1988).

Using information on patterns of genetic variation among coho populations from southern Vancouver Island and the lower coastal mainland, Wehrhahn and Powell (1987) inferred that straying rates from each breeding population were about six successful spawning individuals per generation. For populations with about 1000 breeding adults, this translates into straying rates of approximately 0.5%. Adult straying rates among the five wild and enhanced stocks monitored during this study, with at least 1000 breeding adults, ranged from 0 to 11.1% and averaged 3.7%. This average rate was largely influenced by the relatively high straying rates of Trent River adults. The possibility exists that straying rates for this stock are abnormally high and are not representative of other stocks. Omitting this stock from the calculations leads to an average straying of 1.3%. This figure could represent a maximum straying rate, since it is not known if all strays recovered in various streams would have remained there to spawn successfully. This figure is still higher than the estimate of Wehrhahn and Powell, but the straying rates observed in the present study apply to local populations and are expected to be higher than those based across all stocks covering a wide geographical area. Wehrhahn and Powell estimated that the straying between the lower coastal mainland and southern Vancouver Island populations averaged 50 coho per year. During the present study, only two strays were detected from the mainland, but the area monitored was a small portion of southern Vancouver Island, so the straying estimates of Wehrhahn and Powell cannot be rejected based on the present study.

Quinn and Fresh (1984) estimated straying rates among chinook salmon released from the Cowlitz River hatchery in the Lower Columbia, based on CWT recovery patterns. They found that the adults did not stray substantially (<1.5%) and strays were generally recovered in streams within 30 km of the Cowlitz River. They reported a fourfold difference in the amount of straying between successive year classes, indicating that there can be pronounced year to year changes in straying. Quinn and Fresh found that older age classes tended to stray more than younger ones. Quinn et al. (1991) reported that older chinook salmon (ages 3–5) from the Columbia River also strayed more than younger ones (age 2) and found variation in straying among return years. Lower straying of younger coho was also observed in the present study, indicating that age can have similar influences on straying in different species of Pacific salmon. Quinn et al. speculated that differences in straying rates among age groups might be due to changes in the chemical characteristics of the stream over the years, or to loss of memory as the fish get older.

Quinn and Fresh (1984) noted that high homing proportions were associated with large returns and speculated that social factors strengthen the motivation to home. During the present study, no significant correlation was detected between homing proportions and the number of recoveries to a given stream, but perhaps this is a consequence of the relatively narrow range of recoveries observed during the study. However, insight into the nature of the factor(s) which affect the ability of coho to recognize their home stream can be gained from further examination of the rearing conditions of stocks exhibiting the greatest straying. Rosewall Creek releases consisted of the progeny from distant populations, which were hatchery reared and released from a stream which was not their parental one. This hatchery uses mainly surface water from Rosewall Creek for rearing purposes, but relies heavily on groundwater during June to September when surface flows are insufficient (R. Humphreys, pers. comm.). Fry released in the Millstone and Trent rivers for enhancement purposes were also reared at hatcheries located at some distance from their stream of origin, using nonnatal water sources. Thus, it is hypothesized that the process of exposing fry to groundwater or foreign (nonnatal) water sources for a prolonged period during the rearing stage increases the amount of straying exhibited upon return. In support of this hypothesis, the large fraction of the strays detected in the Puntledge River consisted of coho reared at the hatchery and released in the headwaters of the adjacent river (Tsolum River) for colonization purposes.

It seems reasonable to suspect that some of the year to year variation in straying observed within a small region must be attributed to changes in the tagging and production regimes at various hatcheries and in the streams themselves. However, biological and physical factors should play important roles as well. The relative abundance of strays among the Trent River adult population was highest in 1986 and 1987 when a prolonged drought period affected the upstream migration in all streams in the region lacking flow controls. Insufficient flows may inhibit the ability of early maturing coho to move upstream and may force them to seek alternative refuges and spawning locations. Run timing in streams lacking flow controls is largely influenced by the level of precipitation early in the season. This suggests a mechanism by which delays in run timing could be related to increases in straying. Trends in predicted homing proportions reported earlier also suggested that early runs were more likely to stray than later runs, which could be an adaptation of early runs to cope with the greater probability of insufficient flows early in the season.

It is puzzling that Trent River differs markedly from other streams in terms of its ''attractiveness'' to strays. Presumably, the proximity of the Trent and Puntledge rivers is partly responsible for the large number of Puntledge River strays found in the Trent River. However, the geographical location of the Trent River does not explain the relatively large number of strays from distant locations, such as from across the Strait of Georgia (Porpoise Bay) and the Lower Fraser (Capilano River). The Trent River does have a larger and more continuous discharge than other small streams and a relatively large estuary. Strays might be attracted more readily to larger rivers than to small streams. In support of this hypothesis, hatchery strays were observed to account for ~30% of the Tsable River escapement in 1986 (R. Hurst, pers. comm.), which is physically similar to the Trent River and located just 10 km south of it. Large numbers of strays were also detected in larger streams such as the Big Qualicum River and the Puntledge River. The number of strays found in the Quinsam River was negligible, but the river location (at the edge of the study area) and the absence of tagging in an adjacent stream could have played a role here.

The emigration of coho observed at the Trent River following a period of stream residency has been observed in other coastal streams where mark–recapture operations were conducted (N. Schubert, pers. comm.). The large emigration of adults from the Trent River in 1986, coupled with their return to the Puntledge River hatchery, suggests that many of the coho entering the Trent River are simply exhibiting ''proving'' behavior (Ricker 1972) and may not be committed to spawning there. Whether or not the number of coho proving a stream is related to particular attributes of the stream or the size of the stray populations is not known. No strays were ever detected at French Creek, Black Creek, and Millstone River, although they are located in the proximity of public hatcheries (at Big Qualicum, Quinsam, and Nanaimo rivers) which had substantial runs. The Trent River fence is the only one located in intertidal waters, and the number of strays detected might have been lower if the fence had been placed further upstream. Alternatively, it may be that smaller streams have a more characteristic odor (Hasler and Scholz 1983) which allows coho to distinguish them more easily than the Trent River, which has unusually clear water and comparatively little organic debris. Perhaps this type of water does not provide strong olfactory cues, which may mislead coho that enter this stream. Increases in discharge rates may provide stronger cues, allowing coho to distinguish this system from their own and induce them to move out, as was observed during this study when floods occurred.

The lack of significant numbers of hatchery strays in the escapements of French and Black creeks indicates that the level of interbreeding with hatchery coho from neighboring public hatcheries is not large in all natural systems located in proximity to these facilities. However, the extent of the contribution of hatchery strays to the Trent River escapement, coupled with the apparent hybridization, suggests that there may be considerable year to year variability in the stock composition of its spawning population. The magnitude of the genetic introgression could not be quantified during the present study, but the evidence presented suggests that it could be considerable and should be taken into account for a proper evaluation of the genetic consequences of releasing artificially propagated fish into the wild (Waples et al. 1990).

Although the overall level of straying observed during the present study tended to be small, some stocks exhibited a considerable amount of straying in some years. In such cases, not accounting for the strays would lead to erroneous estimates of exploitation and survival rates. Assume for instance that 20 000 coho smolts are tagged during their migration. Of these 2000 tagged adults survive and are intercepted in the fishery. Half of the 800 coho that escaped to the stream are tagged and lack an adipose fin. Assume that all 400 tagged coho in the escapement originated from that stream and that another 80 tagged coho from this stock escaped to other streams. The exploitation rate for this stock, usually estimated from the ratio of catch to catch plus escapement, would be 80.6% if all strays were accounted for and 83.3% if strays were not accounted for. So not accounting for the 20% stray rate would have resulted in positively biased estimates of exploitation rate. Additional simulations conducted with the same level of straying indicated that the magnitude of the bias increases with decreasing exploitation rates. Further simulations also indicated that the bias increases with increasing straying rates for a given exploitation rate.

The contribution of hatchery strays to a given population must also be taken into account. For the above case, assume that 100 of the 400 tagged coho in the stream were of hatchery (foreign) origin, so the actual exploitation rate becomes 84.3%. If the investigator cannot account for the contribution of hatchery strays in the tagged coho escapement (in this case, 25%), then the estimated exploitation rate would be negatively biased. Of course, sampling deficiencies could further compound the problem and increase the amount of bias. Additional simulations revealed that under realistic conditions such as encountered during this study, the discrepancy between the actual and the biased estimates of exploitation can exceed 10% if straying or stray contributions are not accounted for.

Estimates of survival rates, obtained from the ratio of total recoveries (catch plus escapement) to total release, can also be biased if strays are not accounted for. For the case described above, the actual survival rate would be 12.4% if straying to other streams is accounted for and 12.0% if such straying is unaccounted for. Thus, not accounting for straying (or stray contributions) has a relatively greater influence on exploitation estimates than on survival estimates. However, coho smolt to adult survival rates are usually not as variable as exploitation rates, so the small bias in survival rate estimates can still be relatively important. For these reasons, it is recommended that surveys be designed to account for straying when coho populations are subject to coded-wire tagging and escapement enumeration for stock assessment purposes.

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Validation Studies of Daily Increment Formation for Larval and Juvenile Summer Flounder, *Paralichthys dentatus*

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The increment deposition rate in sagittal otoliths of larval and juvenile summer flounder, *Paralichthys dentatus*, was influenced by growth rate. Larvae of known age showed less-than-daily increment deposition during slow growth (0.1 mm·d^{-1}), but increment deposition appeared daily after the growth rate increased to 0.4 mm·d^{-1}. There was no significant difference between counts from larval otoliths made by either scanning electron microscopy or light microscopy. Juveniles collected in the field that were tetracycline marked and held in the laboratory at elevated temperatures (≈15°C) showed significantly faster growth rates (0.3 mm·d^{-1}) and daily increment depositon rates compared with growth rates (−0.03 mm·d^{-1}) and less-than-daily increment deposition rates at ambient temperature (1–11°C). Tetracycline-marked juveniles, held in cages in a subtidal creek, also showed daily increment deposition. Scale circuli were not deposited daily in the juveniles examined. It appears that daily aging of the youngest *P. dentatus* larvae is problematic, but after settlement in estuarine nursery areas, increment formation occurs daily.

Le taux de dépôt des stries d'accroissement dans l'otolithe (sagitta) des larves et des juvéniles de cardeau d'été, *Paralichthys dentatus*, était influencé par le rythme de croissance. Chez les larves d'un âge connu, le taux de dépôt n'était pas mesurable quotidiennement pendant la période de croissance lente (0,1 mm·d^{-1}), mais il était mesurable quotidiennement après augmentation du rythme de croissance (0,4 mm·d^{-1}). Il n'y avait pas de différence notable dans le dénombrement des stries sur les otolithes de larves entre la microscopie électronique à balayage et la microscopie optique. Les juvéniles capturés sur place et marqués à la tétracycline puis gardés au laboratoire à température élevée (≈15°C) montraient un rythme de croissance (0,3 mm·d^{-1}) et un rythme quotidient de dépôt des stries d'accroissement nettement supérieures par rapport au rythme de croissance (−0,03 mm·d^{-1}) et un taux de dépôt inférieur au rythme quotidien à température ambiante (1–11°C). Les juvéniles marqués à la tétracycline, maintenus dans des cages dans un ruisseau infratidal, présentaient aussi un rythme quotidien de dépôt de stries d'accroissement. Les anneaux des écailles ne se déposaient pas quotidiennement chez les juvéniles examinés. Il apparaît problématique de mesurer quotidiennement l'âge des larves les plus jeunes de *P. dentatus*, mais après installation dans les nourriceries estuariennes, la formation des stries d'accroissement se produit quotidiennement.

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Daily age determination from otolith increments has been successfully applied to the larvae and juveniles of many fishes from a variety of habitats (Campana and Neilson 1985; Jones 1986) and has recently been extended to scales (Szedlmayer et al. 1991). However, validation attempts have not always been successful (Geffen 1986; Lough et al. 1982; Davies et al. 1988), and recently, otolith resorption has been shown under stress in goldfish, *Carassius auratus* (Mugiya and Uchimura 1989). Thus, before daily aging can be applied, validation is needed for each species under a variety of conditions. The purpose of the present study was to validate daily aging methods for larval and juvenile summer flounder, *Paralichthys dentatus*, prior to studies of birth date distribution and habitat-specific growth. Several methods were used to test for daily increment deposition based on examination of (1) sagittal otoliths from larvae of known age with light and scanning electron microscopy (SEM), (2) otoliths from wild tetracycline-marked fish undergoing metamorphosis, and (3) tetracycline-marked scales of juveniles.

Methods

Sources of Larvae and Juveniles

Adult *P. dentatus* were collected from Little Egg Inlet, New Jersey, USA, by hook and line in September 1988. The fish were held in a flow-through, 1000-L rectangular tank under natural photoperiod, salinity, and temperature and fed ad libitum every third day with frozen fish and squid. Three males and five females (345–425 mm total length (TL)) were injected in March 1989 with 0.011 mg carp pituitary hormone·g wet weight^{-1} to induce spawning (Smigielski 1975). The resulting larvae were reared in 75-L circular polyethylene tanks under natural photoperiod, salinity, and elevated temperature (≈15°C). Larvae (1–24 d after hatching) were fed wild plankton (63–250 μm) and laboratory-reared rotifers, *Brachionus plicatilis*, at 500·L^{-1}·d^{-1}. One third of the water was replaced every 2 d with filtered (63 μm) seawater. Larvae were removed from the 75-L tanks, 24 d after hatching, and placed in 10-L floating containers (eight containers stocked with 20 larvae each; diameter 270 mm, depth 210 mm, 0.153-mm-mesh bottoms) in a 770-L closed system equipped with a biological filter. After the fish were transferred to floating containers, the diet was changed to *Artemia franciscana* nauplii at $100 \cdot L^{-1} \cdot d^{-1}$, plus 500 wild plankton$\cdot L^{-1} \cdot d^{-1}$. After 32 d posthatch the larvae were offered *A. franciscana* nauplii at $100 \cdot L^{-1} \cdot d^{-1}$.

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Larvae undergoing metamorphosis were collected from Little Sheepshead Creek, New Jersey, with a plankton net (1 m, 1-mm mesh) in the winters of 1988–89 and 1989–90. Juveniles (68–115 mm TL) were collected by trawl on 14–15 June 1989 from Great Bay, New Jersey (for further collection methods, see Szedlmayer et al. 1992).

Otolith Preparation and Increment Counting

Larvae ($N = 55$) were measured live (TL) after anesthetization in 50 mg MS222$\cdot$L seawater^{-1} at 12, 17, 26, 47, and 70 d posthatch and then preserved in 95% ethanol. Larvae from days 12, 17, and 26 were mounted whole in a low-viscosity epoxy resin (Spurr 1969). For older larvae (47 and 70 d), otoliths were removed and mounted in resin. After mounting, sagittal otoliths (or whole larvae) were ground to a thin section using 400- to 600-grit wet–dry sandpaper and 0.3-μm type A alumina on micropolishing cloth (Buehler Inc.). Otoliths from the youngest larvae ($\leq$26 d) were counted whole (grinding was only used to remove excess resin and body material and stopped before otolith penetration), but older larvae and juveniles were ground in the sagittal plane to the primordium before counting. Otolith increments were counted at 1250× magnification using an Olympus BH-2 microscope with attached Ikegami black and white video camera (625-line resolution) and Panasonic PM205AM monitor (1000-line resolution). Increments were counted three times by the same reader from the primordium to the edge on a transect clear of any secondary primordia. Otoliths with <15% agreement among increment counts were rejected. The average increment count was used for subsequent analysis.

After otolith increments were counted under light microscopy, a subsample of otoliths ($N = 20$) from larvae of known age were counted under SEM. All otoliths required grinding for SEM. Repeated examination while grinding allowed precise sagittal midsection preparations of even the smallest otoliths. This method resulted in overgrinding 20% of the time, but all such samples were excluded from further analysis. After grinding, otoliths were etched with 5% EDTA$\cdot H_2O^{-1}$ (by weight) for 30, 45, 60, 70, 90, 120, 130, and 170 s. After each etching, an Anateck LTD sputter coater was used to coat otoliths with a 16-nm thickness of gold–palladium. Individual otoliths were counted on the video screen of an Hitachi S-510 SEM at 4000× magnification. Otolith increment counts were included in the analysis only when clear increments were present from the primordium to the edge. For example, underetching was indicated by smooth flat sections on the otolith and overetching was indicated by eroded sections.

Tetracycline Marking

Metamorphosing larvae and juveniles (size range = 15.6–23.8 mm TL, $N = 52$) collected with plankton nets between 26 December 1988 and 23 January 1989 were marked on 27 January 1989 by immersion in a tetracycline solution for 2 h (750 mg oxytetracycline dihydrate (OTC)$\cdot$L 10% NaCl deionized H_2O^{-1}). Individual fish were reared in 1.5-L glass bowls and fed *A. franciscana* nauplii at $500 \cdot L^{-1}$. Water and food were changed daily. These fish were divided into two groups: one group was reared at elevated water temperature ($N = 23$, ≈15°C) and the other at ambient marsh creek water temperature ($N = 18$, 1–11°C). Fish were sacrificed for otolith samples at 12, 36, and 48 d after marking. All tetracycline-marked otoliths required grinding to the primordium. Increments were counted three times at 1250× magnification with a light microscope from the fluorescent mark (visible as a red band under blue light) to the edge along a transect that was uninterrupted by secondary primordia.

On 12 March 1990, fish (size range = 17.3–21.1 mm TL, $N = 7$) collected 8 March 1990 were marked with 500 mg OTC$\cdot$L seawater^{-1} for 24 h. These fish were reared in the laboratory at ambient temperature under the same feeding regimes as above. After 31 d, fish were sacrificed for otolith examination.

On 11 April 1990, metamorphosing larvae and juveniles (size range = 10.6–14.6 mm TL, $N = 81$) collected between 10 March and 9 April 1990 were marked by immersion in 500 mg OTC$\cdot$L seawater^{-1} for 24 h. Fish were placed in cages (1.0 × 1.0 × 0.4 m, 2-mm-square mesh) in the subtidal portion of a creek that drains into Great Bay, 2 km west of Little Egg Inlet. The bottom mesh was pushed into the substratum so that a layer of sediment covered the bottom of the cages. Fish were stocked at 10–16 fish per cage in seven cages. Fish were retrieved and sacrificed at 14 d (two cages), 21 d (two cages), and 43 d (three cages). Otoliths were prepared and counted as above.

Scale Preparation and Circuli Counting

To examine the relationship between scale circuli formation and daily age, scales of juvenile *P. dentatus* (size range = 68–120 mm TL, $N = 54$) were marked by immersion in 750 mg OTC$\cdot$L seawater^{-1} for 24 h. Fish were held in 190-L outdoor flow-through tanks under natural photoperiod, temperature, and salinity at 8–10 fish per tank. Scale samples were taken from the dorsal side near the caudal peduncle (at least six scales) after anesthetizing the fish with 50 mg MS222$\cdot$L seawater^{-1} at 20, 21, 26, and 31 d after marking. Scales were mounted on glass slides in liquid coverslip (''Flo-tex'', Learner Laboratories) and covered with a glass cover slip. The tetracycline mark was visible as a yellow fluorescent mark under 100-W ultraviolet light. Scale circuli were counted from the fluorescent mark to the edge on the anterior side of the scale at 400× magnification. The highest count among the six scales taken from an individual fish was used for analysis.

Statistical Analysis

A 0.05 level of significance was used in all statistical analysis (*t*-test, paired *t*-test, and analysis of covariance (ANCOVA)). If a null hypothesis was accepted (i.e. 1 increment$\cdot d^{-1}$), before concluding daily increment deposition rates, the power of the test was determined (i.e. power = the probability of not making a type II error) (Rice 1987; Peterman 1990).

Results

Deposition rates of sagittal otolith increments varied with growth rate in the laboratory-reared *P. dentatus*. During early periods of slow growth (1–47 d posthatch, growth rate = 0.1 mm$\cdot d^{-1}$, size range at 47 d = 5.5–7.6 mm TL), the otolith increment deposition rate was less than daily, based on light microscopy (Fig. 1). From 47 to 70 d posthatch, growth rate increased (0.4 mm$\cdot d^{-1}$) and increment deposition appeared daily. Otoliths grew very little (0.7 μm$\cdot d^{-1}$) from day 12 to 47 but increased in size substantially (11.5 μm$\cdot d^{-1}$)

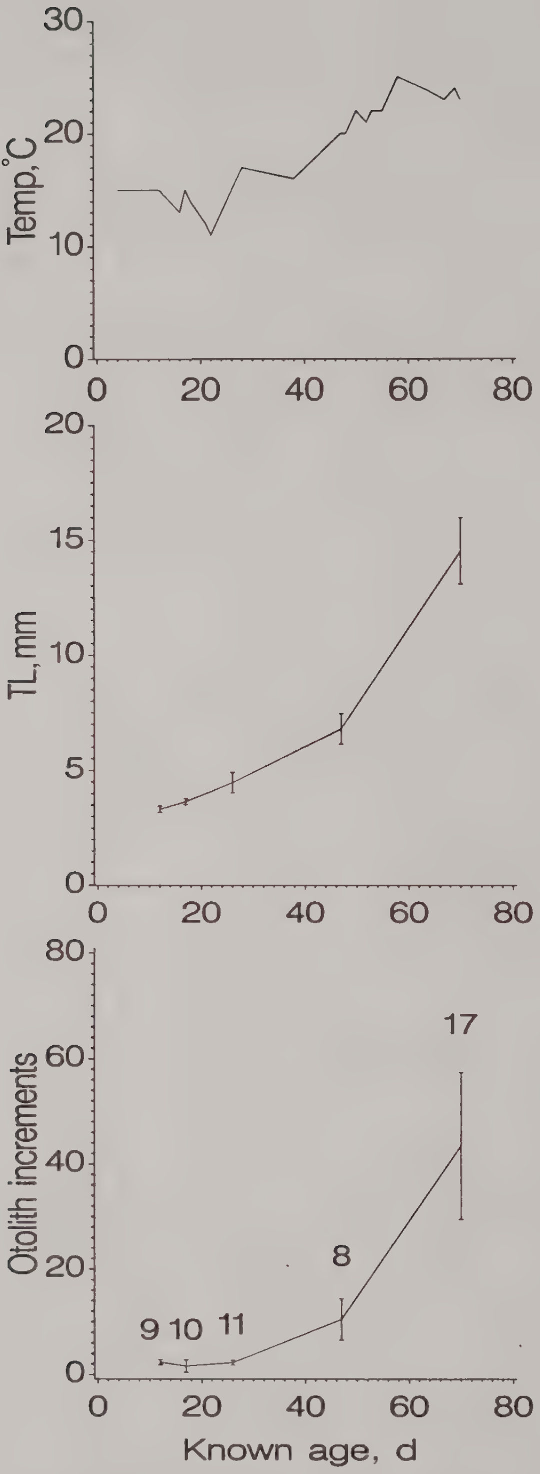

FIG. 1. Otolith increment counts (light microscope) and growth (TL) of larval summer flounder of known age relative to temperature. (Top) temperature of rearing environment; (middle) mean TL versus age; and (bottom) mean otolith increment counts versus age. Lines connect mean values, vertical bars are SD, and numbers above SD bars = N. Metamorphosis occurred over days 55–80.

TABLE 1. Comparison of otolith increment counts for summer flounder of known age under the light microscope and SEM. A paired t-test showed no significant difference between counts (0.05 level).

Fish	Age (d)	Light	SEM	Change	Etching time (s)
1	17	0	0	0	71
2		2	0	−2	45
3		0	5	+5	30
4		3	3	0	30
5		2	1	−1	30
6		0	2	+2	30
7	70	48	43	−5	130
8		72	61	−11	130
9		51	43	−8	130
10		52	59	+7	130
11		42	33	−9	130
12		47	54	+7	130
13		31	47	+16	130
14		43	22	−21	130
15		32	38	+6	130
16		51	35	−16	130

from day 47 to 70. Mean ± SD otolith radii (from the maximum diameter) at 12, 17, 26, 47, and 70 d were 11.6 ± 1.1, 12.4 ± 1.6, 13.9 ± 1.2, 23.6 ± 7.1, and 156.1 ± 95.1 μm, respectively. Examination with SEM of a subsample of otoliths from laboratory-reared fish failed to show consistent differences in increment counts compared with those counted with light microscopy (no significant difference, paired t-test, Table 1). Figure 2 shows light microscope and SEM photographs of a typical otolith from a larva 17 d after hatching. Three increments were counted with both techniques.

Both fluorescent marking methods (750 mg of OTC, 10% NaCl freshwater, 2-h exposure; 500 mg of OTC, seawater, 24-h exposure) left clear visible time marks on all otoliths and scales. The appearance of marks on *P. dentatus* otoliths differed from other studies (Sogard 1991) in that visible fluorescence was red under blue-light excitation instead of yellow under UV light. No mortality was observed due to exposure to the OTC solutions.

The increment deposition rate was also influenced by growth rates in tetracycline-marked fish that were collected with plankton nets and held in the laboratory (Fig. 3). Otolith increments from tetracycline-marked fish held at elevated temperature (≈15°C) showed a daily deposition rate up to 48 d after marking (1.1 increments·d^{-1}, not significantly different from 1 increment·d^{-1}, $N = 22$, $t = 0.39$, power = 0.86. However, in fish held at ambient temperature the deposition rates were significantly less than daily (0.7 increments·d^{-1}, $N = 19$, $t = 2.45$). In addition, the deposition rates at ambient and elevated temperatures were significantly different from each other (ANCOVA, $F = 62.31$). Mean growth rate at ambient temperature (−0.03 mm·d^{-1}, $N = 31$) was significantly lower (ANCOVA, $F = 14.16$) than that observed at elevated temperature (0.31 mm·d^{-1}, $N = 45$). These slower growth rates are consistent with natural events because recruitment of metamorphosing larvae into the estuary occurs during the winter (Able et al. 1990; Szedlmayer et al. 1992).

Otolith increments of tetracycline-marked juveniles held in the subtidal portion of a creek (Fig. 4) were deposited daily up to 43 d after marking (1.1 increments·d^{-1}, not significantly different from 1 increment·d^{-1}, $N = 41$, $t = 1.0$, power = 0.93). The mean growth rate for fish held in the subtidal creek (0.4 mm·d^{-1}, $N = 122$) was significantly faster

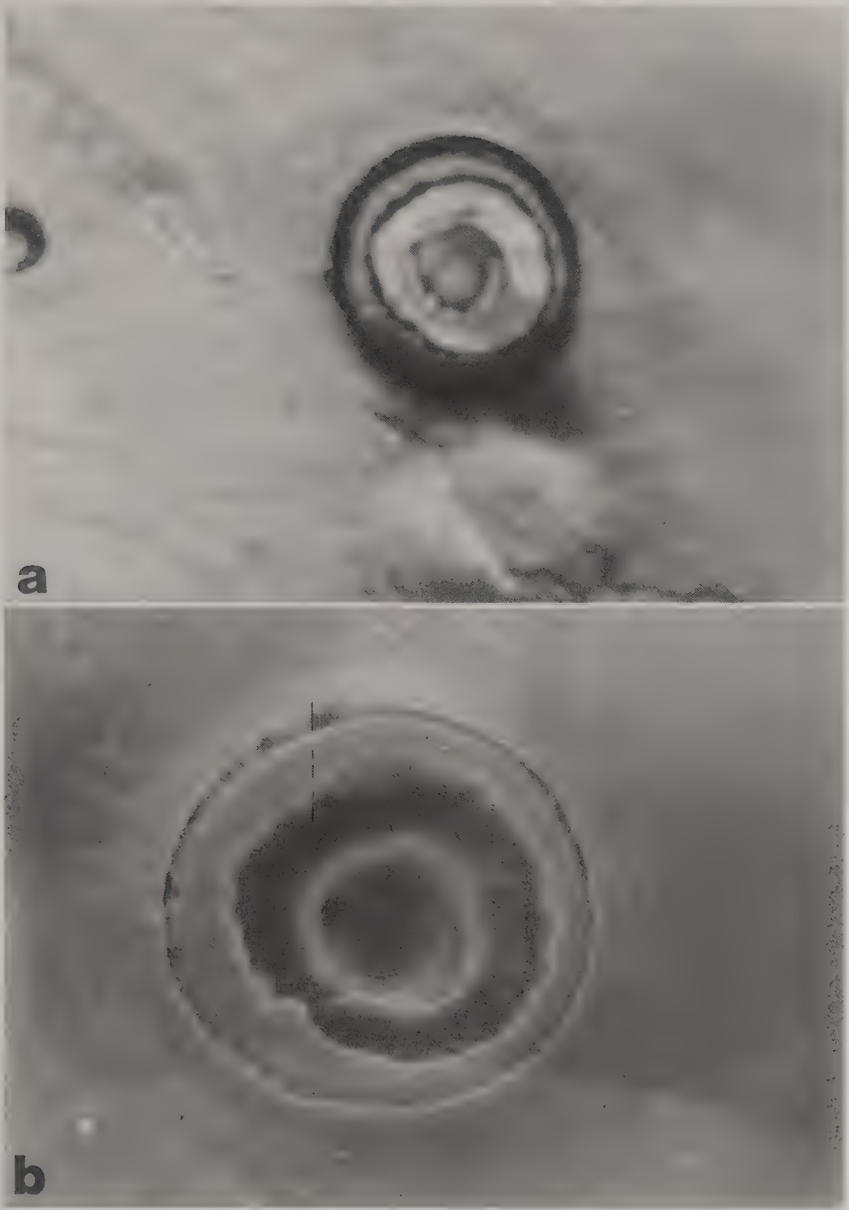

FIG. 2. Photographs of an otolith (diameter = 24 μm) from a 17-d-old larval summer flounder reared in the laboratory. (a) Light microscope photograph at 1250× magnification; (b) SEM photograph of the same otolith at 4000× magnification.

than those recorded for fish held at both ambient and elevated temperatures in the laboratory (ANCOVA, $F = 14.98$), despite similar temperatures in the creek and during the earlier laboratory treatments at elevated temperatures (Fig. 3 and 4).

Scale circuli of juvenile *P. dentatus* (68–115 mm TL) were apparently not deposited daily (Table 2). In all cases, there were significant differences between circuli count and number of days after marking. In addition, as days from marking increased, the difference between circuli count and days also increased. Thus, a constant correction factor for scale circuli could not be estimated. In addition, *P. dentatus* scale circuli were difficult to count because a single circulus often split into two circuli.

Discussion

Deposition rates of sagittal otolith increments of *P. dentatus* varied with age (size) and growth rates. However, a basic assumption in determination of in situ growth rates of larval and juvenile fishes with otoliths is that otolith increment deposition rates are daily and independent of somatic growth. Although many studies have shown daily deposition rates of otolith increments (see reviews in Campana and Neilson 1985; Jones 1986), such validation usually does not reveal information on the relationship between growth and deposition rate. Other studies (Geffen 1982; Rice et al. 1985; Sogard 1991; Titus and Mosegaard 1991) have also shown less-than-daily

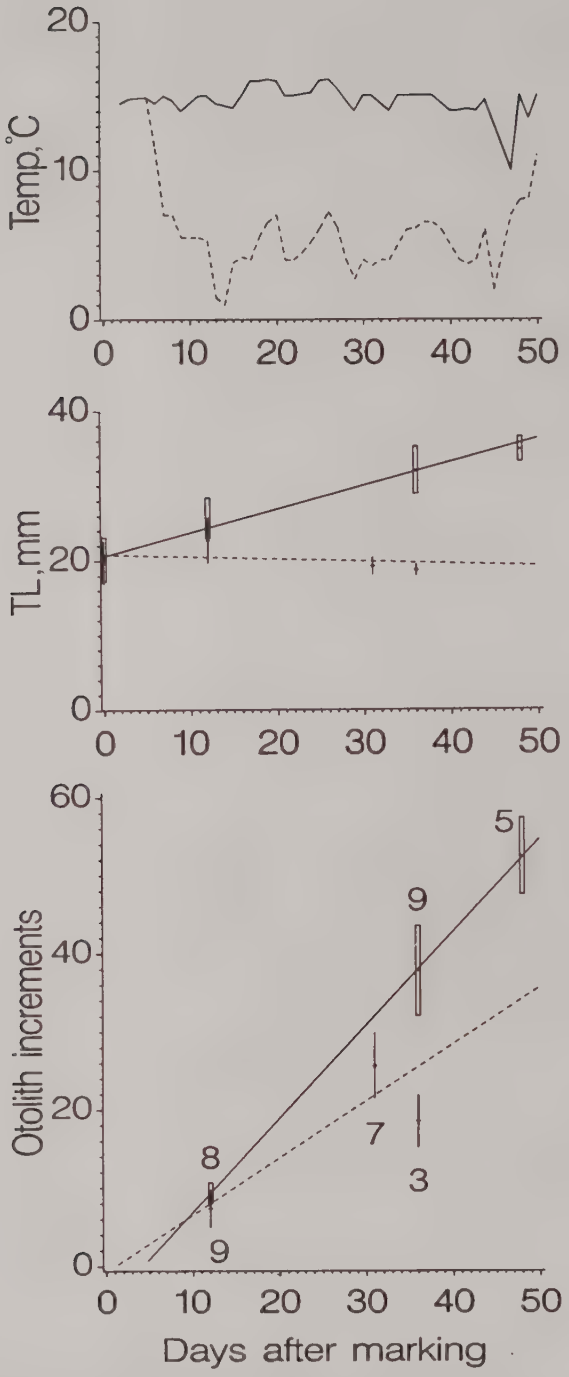

FIG. 3. Otolith increment counts (light microscope) and growth (TL) after tetracycline marking of juvenile summer flounder held in the laboratory. Broken lines = ambient water temperature; solid lines = elevated water temperature. Horizontal bars are means, vertical bars are SD, lines are linear regressions, and numbers above SD bars = N. (Top) Water temperature in the laboratory; (middle) growth – ambient temperature ($Y = -0.03x + 20.8$, $r^2 = 0.02$, $N = 31$) and growth – elevated temperature ($Y = 0.31x + 20.5$, $r^2 = 0.80$, $N = 45$); and (bottom) increment count – ambient temperature ($Y = 0.7x - 0.74$, $r^2 = 0.72$, $N = 19$) and count – elevated temperature ($Y = 1.1x - 5.0$, $r^2 = 0.95$, $N = 22$, power = 0.86).

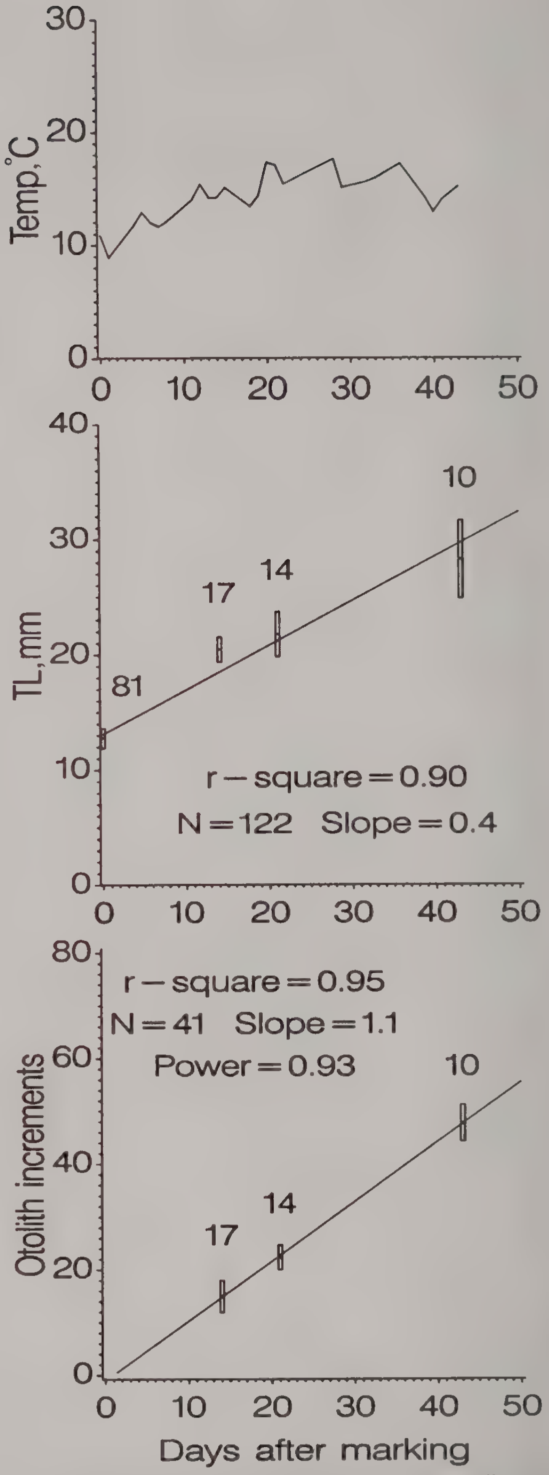

FIG. 4. Otolith increment counts and growth, after tetracycline marking, of caged juvenile summer flounder held in a subtidal creek. Horizontal bars are means, vertical bars are SD, lines are linear regressions, and numbers above SD bars = N. (Top) Temperature in the creek where cages were located; (middle) growth rate of fish in cages ($Y = 0.4x + 13.1$, $r^2 = 0.90$, $N = 122$); (bottom) increment deposition rate of fish in cages ($Y = 1.1x - 1.1$, $r^2 = 0.95$, $N = 41$, power = 0.93).

TABLE 2. Mean scale circuli counts ($C \pm$ SD) after tetracycline marking for summer flounder held in outdoor flow-through tanks. N = number of fish examined, TL = mean total length ± SD, D = days after marking. *Significant difference between D and C was detected by a t-test (0.05 level). Range = range in total length of fish examined.

Date	N	TL ± SD (mm)	D	C ± SD	t-test	Range (mm)
15–16 June 1989	53	91.7 ± 10.8	0	Marked		68–105
5 July 1989	29	109.1 ± 2.5	20	23.1 ± 4.0	*	86–136
6 July 1989	24	108.6 ± 15.0	21	22.5 ± 6.5	*	79–137
5–6 July 1989	53	108.8 ± 13.6	0	Marked		79–137
1 Aug. 1989	37	128.4 ± 20.2	26	29.8 ± 9.9	*	92–176
6 Aug. 1989	16	125.6 ± 14.3	31	25.9 ± 7.6	*	103–155

deposition rates that were related to growth rates. A major criticism of less-than-daily deposition rates has been that increment widths are sometimes too narrow to detect with the limited resolution of the light microscope (Campana et al. 1987; Bailey and Stehr 1988). In our examination of *P. dentatus* otoliths at the higher magnification possible with SEM (4000 ×), there was no significant difference from counts made with light microscopy (Table 1). To eliminate the possibility that etching times prior to SEM might influence counts, we examined otoliths etched for several different periods of time and only included those that revealed clear increments from the primordium to the edge. In contrast, other studies suggested that increased resolution of SEM qualitatively increased increment counts in striped bass, *Morone saxatilis* (Jones and Brothers 1987), while others showed a complete breakdown of increment formation at slow growth rates in sea bass, *Dicentrarchus labrax*, with SEM (Morales-Nin 1987).

Our interpretation of less-than-daily deposition for *P. dentatus* otoliths is also supported by the observation that otoliths from the younger *P. dentatus* (≤17 d) had similar diameters (≈20–25 μm) and the typical "core" structure, i.e. primordium with two or three large (width ≈5 μm) increments, that has been shown for other species including walleye pollock, *Theragra chalcogramma* (Nishimura and Yamada 1988); Antarctic ice fish, *Trematomus newnesi* (Radtke et al. 1989); Japanese eel, *Anguilla japonica* (Tabeta et al. 1987); Atlantic cod, *Gadus morhua* (Radtke 1989); plaice, *Pleuronectes platessa* (Karakiri and von Westernhagen 1989; Al-Hossaini et al. 1989); dolphin, *Coryphaena hippurus* (Uchiyama et al. 1986); and two flounder species, *Rhombosolea tapirina* and *Ammotretis rostratus* (Jenkins 1987). Thus, we feel that our observation of less-than-daily increment deposition rates for premetamorphic larval *P. dentatus* was not the result of resolution problems with light microscopy.

A more likely explanation is that increment deposition in *P. dentatus* was either delayed or occurred at a lower rate during periods of slow growth. A similar interpretation has been reported for Atlantic herring, *Clupea harengus*, where daily deposition was delayed by as much as 22 d posthatch (Lough et al. 1982; Geffen 1982). Other species showing less-than-daily increment deposition rates at slower growth rates include walleye pollock (Bailey and Stehr 1988); brown trout, *Salmo trutta* (Titus and Mosegaard 1991); and winter flounder, *Pleuronectes americanus* (previously *Pseudopleuronectes americanus*) (Sorgard 1991). In addition, other studies have demonstrated that colder temperatures were correlated with reduced increments counts. For example, green sunfish, *Lepomis cyanellus*, ceased daily increment production at temperatures below 10°C (Taubert and Coble 1977), and no increment formation occurred in sockeye salmon, *Oncorhynchus nerka*, reared below 5°C whether fed or starved (Marshall and Parker 1982). Thus, at least for some species, growth rate apparently affects otolith increment deposition rates, and we suggest that it would be difficult to accurately age *P. dentatus* larvae with sagittal otoliths during periods of slow growth associated with winter temperatures.

In contrast, after the completion of metamorphosis when *P. dentatus* are typically experiencing warmer spring and summer temperatures in estuarine nursery areas (Able et al. 1990; Szedlmayer et al. 1992), deposition of otolith increments occurred daily. In three groups of fish, (1) laboratory reared, (2) field caught, tetracycline marked, and held in heated laboratory aquaria, and (3) field caught, tetracycline marked, and field caged, we validated daily increment deposition rates (Fig. 1, 3, and 4). However, further information is needed before applying daily aging methods to this species because of the unknown number of days prior to metamorphosis. One approach that may prove useful would be comparisons of increment width and pattern with life stage and growth rate. For example, Victor (1982) showed a distinct mark on the otoliths of bluehead, *Thalassoma bifasciatum*, that corresponded to the settlement of the planktonic larva onto reef habitat. Settlement marks were also identified in several other species: plaice (Al-Hossaini et al. 1989); starry flounder, *Platichthys stellatus* (Campana 1984); and winter flounder (Sogard 1991). A similar settlement mark might be expected in *P. dentatus*, as it moves from a pelagic oceanic existence to a benthic estuarine habitat (Able et al. 1990; Szedlmayer et al. 1992). If a settlement mark can be identified on the otoliths, the post-metamorphic age of juvenile *P. dentatus* may be determined during their estuarine nursery period.

Szedlmayer et al. (1991) reported evidence for daily circuli deposition in scales of weakfish, *Cynoscion regalis*. However, this was not the case for *P. dentatus*. Although scale circuli were numerous, we were not able to validate the method for this species.

In summary, larvae *P. dentatus* did not show daily increment deposition during larval development, but after metamorphosis, daily deposition does occur. Thus, daily aging methods may be useful for juvenile *P. dentatus* from estuarine nurseries. Critical to future studies would be the testing of many different growth rates to determine if deposition rate is linearly related to growth rate or if some lower growth threshold controls daily increment deposition. For other species, even if daily increments have been validated, we still need to investigate the relationship between growth and deposition rate under less-than-optimal conditions.

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Genetic Protein Variation in Natural Populations of Atlantic Salmon (*Salmo salar*) in Scotland: Temporal and Spatial Variation

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Levels of temporal and spatial genetic heterogeneity within and among Atlantic salmon (*Salmo salar*) populations in Scotland were assessed through starch-gel electrophoretic analysis of variation at 30 protein-coding loci. Over three year-classes the level of temporal variation within populations was generally nonsignificant, although significant heterogeneity among year-classes was found at some loci at some locations. Hierarchical analysis of gene diversity showed that 97.6% of the genetic diversity was found within single sites, with the remaining diversity distributed among sites. While there was no evidence of subpopulation differentiation among a number of samples of Atlantic salmon from the River Tweed system, significant genetic heterogeneity was observed among samples from different river systems within Scotland. Comparison of the levels of among-population heterogeneity in the Atlantic salmon with those found in the closely related brown trout (*Salmo trutta*) over a similar geographical area shows the Atlantic salmon to be relatively genetically homogeneous.

Nous avons mesuré l'hétérogénéité génétique temporelle et spatiale entre populations et à l'intérieur de populations de saumons de l'Atlantique (*Salmo salar*) d'Écosse grâce à une analyse par électrophorèse sur gel d'amidon de la variation à 30 loci qui codent les protéines. Sur trois classes annuelles, la variation temporelle à l'intérieur des populations n'était généralement pas significative, mais on a trouvé une hétérogénéité significative entre les classes annuelles pour certains loci sur des sites donnés. L'analyse hiérarchique de la diversité génétique a montré que 97,6 % de cette diversité se retrouvait sur des sites pris individuellement, le reste étant distribué entre les sites. S'il n'y avait aucune indication de différenciation entre des sous-populations parmi un certain nombre d'échantillons de saumons de l'Atlantique du réseau de la Tweed, on a observé une hétérogénéité génétique significative entre des échantillons provenant de différents bassins hydrographiques d'Écosse. La comparaison de l'hétérogénéité parmi les populations de saumons de l'Atlantique avec les résultats obtenus chez l'espèce proche que constitue la truite brune (*Salmo trutta*) dans une zone géographique similaire montre que le saumon de l'Atlantique présente une relative hétérogénéité génétique.

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The susceptibility of a fish species to loss of genetic diversity through exploitation or abuse by man depends on the manner in which genetic variation is distributed within and among populations of the species concerned (Ryman 1983). Depletion of genetic variability can have effects at many levels: it may affect the fitness of individuals, reduce the potential of populations for adaptation to changing environments, and ultimately alter the pace of longer term evolutionary processes such as speciation or extinction (Soulé and Wilcox 1980). Species with subpopulations that are strongly or completely reproductively isolated are especially prone to rapid loss of genetic diversity. Higher levels of genetic drift occur as a result of the reduction in effective population size associated with subdivision (Maruyama 1970; Denniston 1978). Further reduction of the sizes of these subpopulations may be caused by overfishing or habitat loss resulting in an increased rate of loss of genetic diversity (Thorpe et al. 1981; Wohlfarth 1986). Moreover, small populations are liable to rapid genetic change by the action

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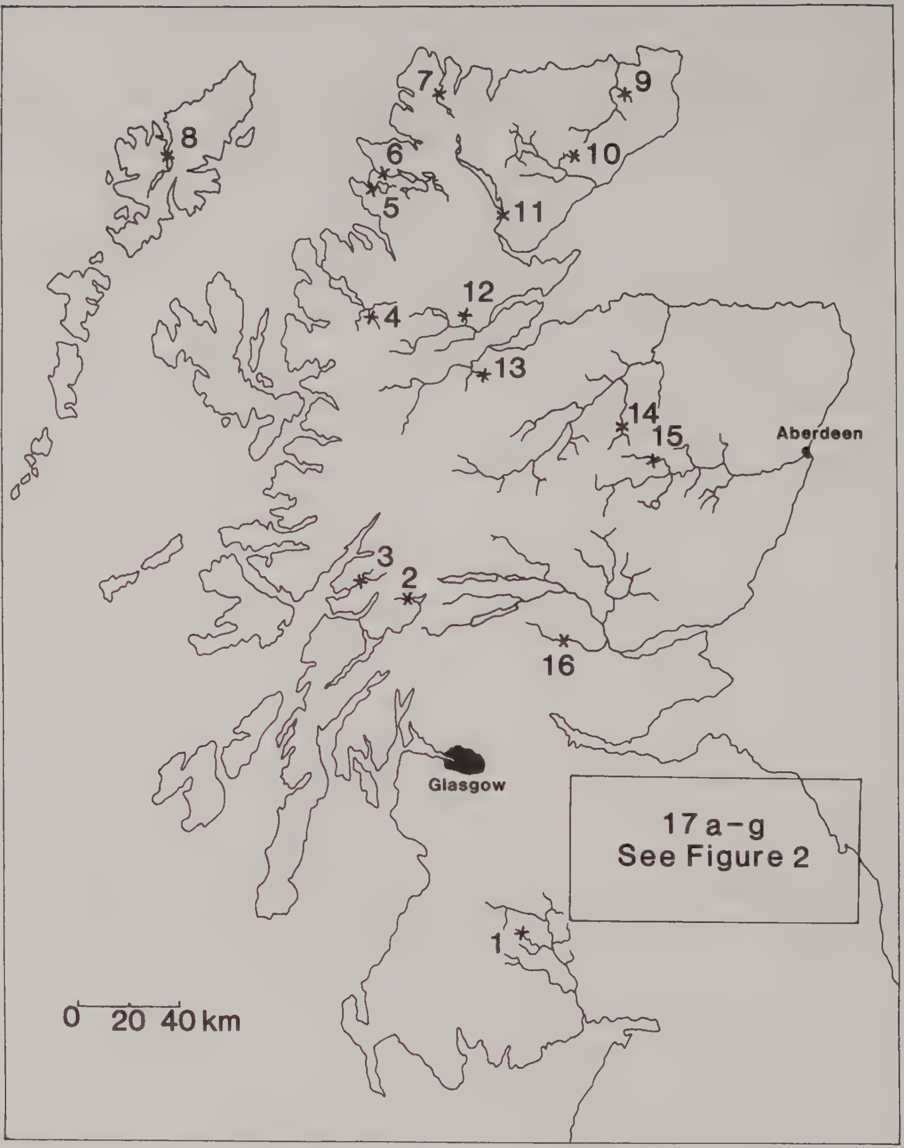

FIG. 1. Locations of sampling sites in Scotland (1987–89). Names of sites identified here by number are given in Table 1.

of gene flow, mediated through natural dispersal or stock transfers by man. An accurate knowledge of population structure is therefore essential for effective fisheries management (Smith and Chesser 1981; Kapuscinski and Lannan 1986; Ryman and Utter 1987).

Analysis of the distribution of genetically based protein variation is one of the main methods for the examination of population structure for fisheries management applications (Ryman and Utter 1987). Numerous studies on the Atlantic salmon (*Salmo salar*) have reported heterogeneity in allozyme frequencies among samples taken from different river systems, and in some cases, from different tributaries within river systems (Cross and Healy 1983; Ståhl et al. 1983; Guyomard 1985; Heggberget et al. 1986; MacCrimmon and Claytor 1986; McElligott 1987; Ståhl 1987; Verspoor 1988b; Crozier and Moffett 1989; Hovey et al. 1989; Koljonen 1989; Ståhl and Hindar 1989). This heterogeneity has generally been interpreted as evidence of reproductive isolation among subpopulations. However, none of the studies cited above has systematically assessed the extent of temporal variation in allele frequencies within putative subpopulations. Demonstration of temporal heterogeneity within populations would require a reappraisal of the conclusions from earlier studies. Much or all of the observed spatial heterogeneity might then be viewed simply as a reflection of temporal fluctuations in allele frequencies around a common mean value at each locus. To test for evidence of population subdivision, temporal and spatial variation must be partitioned to estimate the significance of the spatial variation independently from that of the temporal variation. Analysis of temporal variation in allele frequencies may also provide insight into the relative strengths of the evolutionary forces acting on the genetic variation (Waples 1989).

Genetic protein variation in Atlantic salmon populations in Scotland has not been intensively studied, although a few sam-

TABLE 1. Sampling sites in Scotland (1987–89).

	River system	Tributary	Ordnance survey map reference	Years sampled
1.	R. Nith	R. Scar	NS 759 030	1987, 1988, 1989
2.	R. Orchy	Allt Tolaghan	NN 271 414	1987
3.	R. Creran	Main river	NN 045 480	1987, 1988
4.	R. Ewe	Docherty Burn	NH 046 617	1987, 1988
5.	R. Polly	Main river	NC 074 133	1987, 1988, 1989
6.	R. Kirkaig	Main river	NC 085 193	1988
7.	R. Dionard	Unnamed burn	NC 338 586	1988
8.	R. Grimersta	Main river	NB 214 295	1987, 1988, 1989
9.	R. Thurso	Halkirk Burn	ND 141 598	1987, 1988, 1989
10.	R. Helmsdale	Kinbrace Burn	NC 877 285	1988
11.	R. Shin	Main river	NC 577 068	1988
12.	R. Conon	Black Water	NH 378 708	1988
13.	R. Beauly	Belladrum Burn	NH 503 414	1988
14.	R. Spey	R. Avon	NO 154 092	1987, 1988, 1989
15.	R. Dee	R. Gairn	NJ 295 008	1987, 1988, 1989
16.	R. Tay	Shelligan Burn	NN 945 295	1988
17.	R. Tweed	a. Manor Water	NT 205 324	1987, 1988, 1989
		b. Leader Water	NT 509 533	1988, 1989
		c. Upper main river	NT 111 292	1988, 1989
		d. Eddleston Water	NT 243 427	1988
		e. Tima Water	NT 281 101	1988
		f. Bowmont Water	NT 812 241	1988, 1989
		g. Rule Water	NT 586 118	1988

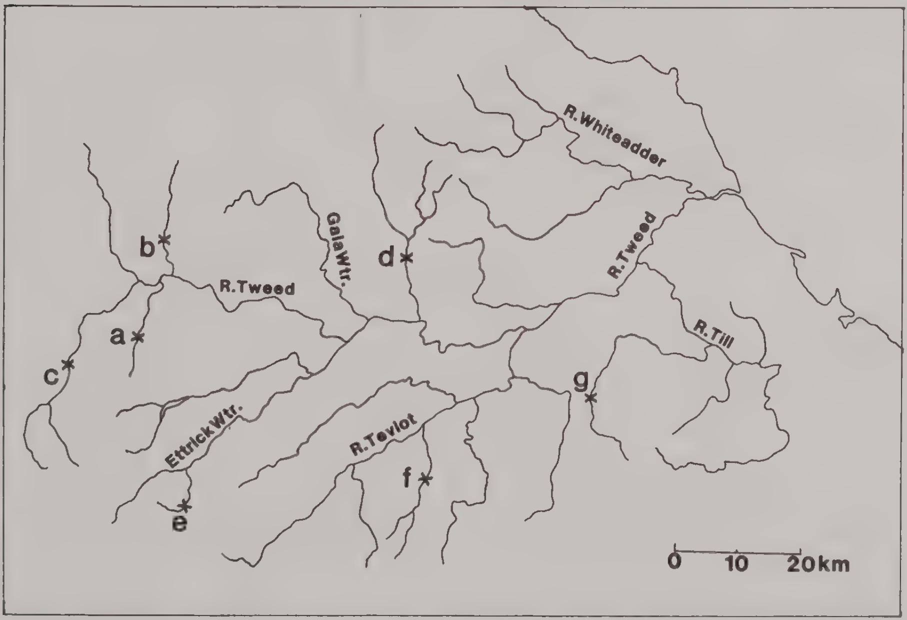

FIG. 2. Locations of sampling sites on the River Tweed system. Names of sites identified here by letter are given in Table 1.

ples from Scottish localities have been included in studies directed towards stock discrimination in mixed-stock fisheries (Cross and Healy 1985; Verspoor 1988a). Because Scotland is an important area for the production of both wild and farmed Atlantic salmon, this represents a major gap in the knowledge of the worldwide distribution of genetic protein variation in the species. In the present study, we examined the spatial distribution of genetic protein variation in natural Atlantic salmon populations from a number of Scottish rivers and the temporal variation in allele frequencies within a subset of these populations.

Materials and Methods

Sampling Sites and Procedures

During the months of July–October in 1987, 1988 and 1989, samples of salmon parr were collected by electrofishing from river systems in Scotland (Fig. 1). A total of 23 sites on 17 separate river systems were sampled over the course of the three years (Table 1). Twelve sites were sampled in each of two or three consecutive years to estimate levels of temporal genetic variation within populations. In order to examine the level of

TABLE 2. Enzyme loci examined in Atlantic salmon in Scotland (1987–89). Names and numbers are those recommended by the International Union of Biochemistry (1984). The buffer systems are fully described in the text. The sample size (N) refers to the number of individual Atlantic salmon examined for genetic variation at those loci over the course of the study. Previously used nomenclature is given in parentheses.

Enzyme name	E.C. No.	Locus	Tissue	Buffer system	Years examined
Aspartate aminotransferase	2.6.1.1	$_s$AAT-4*	L	A	1987–89 (N = 2009)
Adenosine deaminase	3.5.4.4	ADA-2*	L	A	1987
		ADA-3*	L	A	1987 (N = 475)
Alcohol dehydrogenase	1.1.1.1	ADH*	L	B	1987–89 (N = 2009)
Creatine kinase	2.7.3.2	CK-1*	M	B	1987–88
		CK-2*	M	B	1987–88 (N = 1530)
Diaphorase-like enzyme	1.6.-.-	DIA*	L	A	1988 (N = 1055)
Fumarate hydratase	4.2.1.2	FH-1*	M	A	1988
		FH-2*	M	A	1988 (N = 1055)
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH-1*	M	A	1987 (N = 475)
Glucose-6-phosphate isomerase	5.3.1.9	GPI-1,2*	M	B	1987–89
		GPI-3*	M	B	1987–89 (N = 2009)
Iditol dehydrogenase (formerly Sdh)	1.1.1.14	IDDH-1*	L	B	1987–89
		IDDH-2*	L	B	1987–89 (N = 2009)
Isocitrate dehydrogenase (NADP+) (formerly Idh)	1.1.1.42	IDHP-2*	L	A	1987–89
		IDHP-3*	L	A	1987–89 (N = 2009)
Lactate dehydrogenase	1.1.1.27	LDH-4*	L	B	1987 (N = 475)
Malate dehydrogenase	1.1.1.37	$_m$MDH-1*	M	A	1987–89
		$_m$MDH-2*	M	A	1987–89
		$_s$MDH-3,4*	M	A	1987–89 (N = 2009)
Malic enzyme (NAD+)	1.1.1.38 or 1.1.1.39	$_m$ME*	M	A	1987–89 (N = 2009)
Malic enzyme (NADP+) (formerly Me)	1.1.1.40	$_m$MEP-1*	M	A	1987–89
		$_m$MEP-2*	M	A	1987–89
		$_s$MEP-3*	M	A	1987–89 (N = 2009)
Phosphogluconate dehydrogenase	1.1.1.44	PGDH-2*	L	A	1988–89 (N = 1534)
Phosphoglucomutase	5.4.2.2	PGM-1*	L, M	B	1987–89
		PGM-2*	L, M	B	1987–89 (N = 2009)
Superoxide dismutase	1.15.1.1	$_s$SOD*	L	B	1987–89 (N = 2009)

within-river genetic differentiation, seven sites in the River Tweed system in southern Scotland were sampled (Fig. 2).

Sampling sites were limited to areas of intense natural spawning. Sites were chosen where there had been no stocking of Atlantic salmon in recent years, or where supplementary stocking had been carried out using the progeny of adult salmon captured locally. As an exception, a site on the River Polly was chosen because of the river's management history. It has been subject to numerous introductions of salmon parr from at least 17 other river systems, including English and Norwegian rivers (Rt. Hon. E. Davies, Polly Estates, pers. comm.). Moreover, a large smolt production unit is sited on the river where nonnative salmon fry and parr are reported to have escaped.

Approximately 50 salmon parr were collected at each site in each year. Single fish were taken at regular intervals along a 1- to 2-km section of river. Samples were taken in this way in an attempt to ensure that each was an adequate sample of the population at that site and that the same sibling groups were not sampled repeatedly. All samples consisted of 0+ parr only: these were identified and separated from older parr on the basis of fork length (<6 cm). Brown trout (*Salmo trutta*) were identified on the basis of morphological characters (including

TABLE 3. Frequencies of alleles detected in only one sample of Atlantic salmon from Scotland (_*Q0_ indicates a null allele).

Locus	Allele	_N_	No. of alleles	Frequency	Site
ADH*	_*-150_	46	1	0.011	R. Dee, 1987
ADH*	_*-110_	50	2	0.020	R. Kirkaig, 1988
ADH*	_*Q0_	50	3	0.030	Tima Water, 1988
GPI-1,2*	_*20_	46	1	0.011	R. Dee, 1987
GPI-3*	_*80_	47	2	0.021	R. Polly, 1989
IDHP-2*	_*0_	35	3	0.043	Manor Water, 1987
$_s$_MDH-3,4*_	_*115_	49	1	0.010	R. Polly, 1987
$_s$_MDH-3,4*_	_*75_	46	1	0.011	R. Polly, 1988
PGDH*	_*107_	45	1	0.011	R. Dee, 1988
PGM-1*	_*110_	50	2	0.020	R. Thurso, 1987
PGM-2*	_*130_	50	2	0.020	R. Nith, 1988
$_s$_SOD*_	_*85_	49	2	0.020	R. Spey, 1989

body shape, position of eye relative to upper mandible, and pectoral fin length) and excluded from the sample. Upon collection the fish were frozen intact on solid CO_2 and transported to the laboratory where they were stored at −60°C until analysis.

Electrophoresis

Horizontal starch-gel electrophoresis was carried out according to the methods of Taggart et al. (1981). Two buffer systems were used in the electrophoretic analysis: System A, a continuous citrate–aminopropylmorpholine system modified from that described by Clayton and Tretiak (1972) by adjusting the pH to 6.6, and System B, a discontinuous Tris–citrate–borate system modified from that of Ridgway et al. (1970) as described by Verspoor (1988b). Specific staining for enzymes was carried out using the methods of Shaw and Prasad (1970), Harris and Hopkinson (1976), and Abersold et al. (1987).

The loci examined (Table 2) were chosen either because they had previously been shown to be polymorphic in Atlantic salmon or because they could be screened as an adjunct to other analyses. Loci that proved to be monomorphic in samples from 1987 were excluded from the screening programme in later years and replaced by other loci (Table 2). Enzyme loci names and nomenclature follow Shaklee et al. (1990). Adoption of this system involved a number of changes of locus designations from those used in previous studies. These changes are indicated in Table 2.

Brown trout, misclassified as salmon, were identified on the basis of GPI and PGM staining patterns, as were salmon/trout hybrids (Vuorinen and Piironen 1984; Verspoor 1988c). Data from hybrid individuals were not included in the analysis of salmon allele frequencies.

Statistical Analysis

The data were analysed using the BIOSYS-1 computer program of Swofford and Selander (1981). Loci that showed no variation in any of the samples were excluded from the analyses. A locus was considered polymorphic if the frequency of the most common allele was <0.99 in at least one sample. All samples collected at the same site were considered to be replicate samples from a single population. Allele and genotype frequencies were calculated for each locus in each sample by direct counting from phenotypes of individual fish.

The statistical significance of temporal heterogeneity in allele frequencies at single sites was assessed using a modification of the method described by Workman and Niswander (1970). The standardised variance of allele frequencies among samples at single sites (F_{ST} calculated and corrected for sample size as in Wright 1978) was used to produce a χ^2 statistic from the relationship

$$\chi^2 = 2 \cdot N_t \cdot F_{ST}$$

where N_t is the total number of fish sampled at the site. Degrees of freedom associated with this test were $s - 1$, where s is the number of samples taken at the site.

A hierarchical analysis of gene diversity using F_{ST} as the measure of among-sample genetic differentiation was carried out according to the method of Wright (1978). The levels of the hierarchy were defined as individual, year-class, site, river system, and total. The statistical significance of genetic differentiation at each level of the hierarchy was estimated from the F_{ST} statistic as described above.

Results

Observed Genetic Variation

Genetic variation was observed at 14 of the 30 loci examined. The polymorphisms were classified into one of three groups on the basis of their rate of occurrence. Several alleles were detected in one population only (Table 3). Other alleles occurred in more than one population, but in less than half the populations studied (_IDHP-2*160_, _GPI-3*Q0_, and $_s$_MDH-3,4*80_) (Table 4). The _IDHP-2*160_ allele was found in a number of localities on the River Tweed, but in no other river system. Widespread polymorphisms, occurring in >50% of the populations studied, were present at the _IDDH-1*_, _IDDH-2*_, $_s$_AAT-4*_, _IDHP-3*_, and $_m$_MEP-2*_ loci (Table 4). Variation at the _IDDH-2*_ locus, observed in over 75% of the populations studied, could not be resolved adequately and the locus was not included in the statistical analyses.

Temporal Variation in Allele Frequencies

Results of tests on the significance of temporal heterogeneity in allele frequencies at single sites showed a total of nine significant results out of the 65 tests (13.8%) carried out on data from single loci (Table 5). While there appeared to be no bias in the distribution of significant results among loci, the results from the River Polly system were exceptional in that 50% of the tests on single locus data from this site were significant. Moreover, this site was the only one at which the test of heterogeneity over all loci was significant.

TABLE 4. Allele frequencies for occasional and widespread polymorphisms in samples from sites in Scotland (1987–89) (*Q0 indicates a null allele).

	Site	N	IDHP-2*	GPI-3*	$_{m}$MDH-3,4*	IDDH-1*	$_{s}$AAT-4*		IDHP-3*	$_{m}$MEP-2*
			*160	*Q0	*80	*-72	*50	*25	*116	*125
1.	R. Nith									
	1987	50	0	0	0	0.245	0.286	0	0.173	0.510
	1988	50	0	0	0.010	0.420	0.260	0	0.350	0.490
	1989	50	0	0	0	0.380	0.190	0	0.160	0.410
2.	R. Orchy									
	1987	50	0	0	0	0.450	0.120	0.130	0	0.330
3.	R. Creran									
	1987	50	0	0.020	0	0.204	0.130	0	0.040	0.560
	1988	50	0	0	0	0.150	0.180	0	0.020	0.500
4.	R. Ewe									
	1987	47	0	0	0	0.415	0.234	0	0.202	0.277
	1988	42	0	0	0	0.369	0.274	0	0	0.345
5.	R. Polly									
	1987	49	0	0.010	0	0.459	0.306	0	0.133	0.663
	1988	46	0	0	0	0.239	0.185	0.022	0.076	0.370
	1989	47	0	0	0	0.351	0.106	0.053	0.053	0.543
6.	R. Kirkaig									
	1988	50	0	0	0	0.200	0.040	0.050	0.020	0.410
7.	R. Dionard									
	1988	48	0	0	0	0.438	0.073	0.042	0.255	0.281
8.	R. Grimersta									
	1987	50	0	0	0	0.448	0.250	0	0.040	0.670
	1988	48	0	0	0	0.292	0.177	0	0.052	0.617
	1989	50	0	0	0	0.298	0.060	0.010	0.020	0.470
9.	R. Thurso									
	1987	50	0	0	0	0.200	0.235	0	0.143	0.360
	1988	48	0	0	0	0.188	0.135	0.010	0.064	0.542
	1989	49	0	0	0	0.184	0.214	0.010	0.041	0.541
10.	R. Helmsdale									
	1988	50	0	0	0	0.245	0.110	0.010	0.010	0.400
11.	R. Shin									
	1988	50	0	0	0	0.210	0.080	0.050	0.020	0.610
12.	R. Conon									
	1988	50	0	0	0	0.153	0.200	0.010	0	0.490
13.	R. Beauly									
	1988	48	0	0	0	0.302	0.167	0	0.177	0.396
14.	R. Spey									
	1987	48	0	0	0	0.348	0.096	0.106	0.141	0.469
	1988	50	0	0	0.010	0.408	0.130	0.070	0.090	0.540
	1989	49	0	0	0	0.281	0.051	0.031	0.112	0.500
15.	R. Gairn									
	1987	46	0	0	0	0.261	0.174	0.033	0.141	0.370
	1988	45	0	0	0.011	0.267	0.044	0.078	0.111	0.389
	1989	50	0	0	0.010	0.300	0.150	0.010	0.112	0.190
16.	Shelligan Burn									
	1988	46	0	0	0	0.167	0.217	0.043	0.054	0.413
17a.	Manor Water									
	1987	35	0.014	0	0.029	0.229	0.200	0	0.071	0.357
	1988	44	0	0	0	0.330	0.202	0	0.070	0.386
	1989	44	0	0	0	0.284	0.170	0.023	0.057	0.364
17b.	Leader Water									
	1988	48	0	0	0.083	0.394	0.250	0.010	0.135	0.531
	1989	41	0	0	0	0.305	0.232	0	0.085	0.439
17c.	Upper R. Tweed									
	1988	48	0	0.031	0.042	0.375	0.135	0.021	0.083	0.479
	1989	50	0	0	0.040	0.290	0.180	0	0.130	0.410

TABLE 4. _(Concluded)_

Site	N	_IDHP-2*_ _*160_	_GPI-3*_ _*Q0_	$_{m}$_MDH-3,4*_ _*80_	_IDDH-1*_ _*-72_	$_{s}$_AAT-4*_ _*50_	 _*25_	_IDHP-3*_ _*116_	$_{m}$_MEP-2*_ _*125_
17d. Eddleston Water									
1988	44	0	0	0	0.261	0.239	0	0.151	0.386
17e. Tima Water									
1988	50	0	0	0	0.439	0.200	0	0.040	0.510
17f. Bowmont Water									
1988	50	0.122	0	0	0.380	0.120	0	0.120	0.340
1989	49	0.082	0	0	0.204	0.122	0	0.082	0.418
17g. Rule Water									
1988	50	0.030	0	0	0.190	0.260	0	0.153	0.320

Hierarchical Analysis of Gene Diversity

Data for the River Polly site were excluded from any further analyses on the basis of the high levels of temporal variation in allele frequencies observed and because of the stocking history of the river. The mean sampling variance associated with allele frequency estimates at many loci was much greater than the observed variance in allele frequencies over all sites at these loci. Therefore, data on the frequencies of the _ADH*-150_, _*-110_, _*Q0_, _GPI-1,2*20_, _GPI-3*80_, _IDHP-2*0_, $_{s}$_MDH-3,4*115_, _*75_, _PGDH*107_, _PGM-1*110_, _PGM-2*130_, and $_{s}$_SOD*85_ alleles (Table 3) and on the _GPI-3*Q0_ allele (Table 4) were excluded from the hierarchical analysis of gene diversity. Of the remaining loci, only the $_{s}$_AAT-4*_ locus displayed more than two segregating alleles. Frequencies of the two rarer alleles (_*50_ and _*25_) at this locus were pooled to avoid problems associated with averaging F_{ST} values over multiple alleles (Weir and Cockerham 1984; Long 1986).

Hierarchical analysis of gene diversity showed that 96.4% of total diversity was accounted for by differences among individuals within year-classes at single sites (Table 6). Differences among year-classes within sites comprised a further 1.2% of total diversity. An overall test of significance based on the F_{ST} value for this level of the hierarchy showed that the differences between year-classes within localities were not significant ($F_{ST} = 0.012$, $\chi^2 = 48.0$, df = 38, $P > 0.05$). Differences among populations in Scotland represented 2.3% of the total gene diversity within the region. Of this, 0.7% was attributable to differences among sites within a river system (based on the River Tweed), and 1.6% was due to differences among rivers. The differences among sites within the Tweed were not significant ($F_{ST} = 0.007$, $\chi^2 = 8.85$, df = 6, $P > 0.05$). Differences among river systems, however, were highly significant ($F_{ST} = 0.016$, $\chi^2 = 64.3$, df = 15, $P < 0.001$).

Discussion

In general, the pattern of protein variation in Scottish populations was similar to that found previously in other areas of Europe. There were four almost ubiquitous polymorphisms (at $_{s}$_AAT-4*_, _IDHP-3*_, _IDDH-1*_, and $_{m}$_MEP-2*_) and a number of other polymorphisms with variant alleles at low frequencies in one or a few populations (Cross and Healy 1983; Guyomard 1985; Heggberget et al. 1986; McElligott 1987; Ståhl 1987; Crozier and Moffett 1989; Hovey et al. 1989; Koljonen 1989; Ståhl and Hindar 1989). Alleles segregating at the $_{s}$_AAT-4*_, _IDHP-3*_, _IDDH-1*_, and $_{m}$_MEP-2*_ loci are common to Atlantic salmon populations from all parts of the species range (Ståhl 1987; Verspoor 1988a, 1988b; Koljonen 1989). Similarly, the four $_{m}$_MDH-3,4*_ alleles found in Scotland (_*115_, _*100_, _*80_, and _*75_) have been reported from both Europe and North America (Ståhl 1987; Verspoor 1988a, 1988b). It is interesting to note that both the $_{m}$_MDH-3,4*115_ and _*75_ alleles were found in the River Polly samples but were not detected in any of the other Scottish populations. This may be a consequence of the many introductions into the River Polly system of salmon originating from other areas.

Overall genetic differences among year-classes were not significant, although significant differences in allele frequencies among year-classes occurred at some loci in some populations. The proportion of significant results on tests at single loci (13.8%) was greater than expected at the nominal level (5%). However, it has been pointed out that while conventional tests of homogeneity (e.g. χ^2 or _G_-tests) are designed to take statistical sampling error into account, they do not allow for the processes of genetic sampling between generations, particularly genetic drift (Wilson 1980; Waples 1989). Tests that do consider the effect of genetic drift require estimates of the effective sizes of the populations involved (Waples 1989). These estimates are not available for the populations studied here. Therefore, a conventional test with a 5% level of significance has been used to estimate temporal variation in this study, not least because it allows comparisons with other studies.

The level of temporal variability reported here is consistent with results from previous studies on Atlantic salmon (Crozier and Moffett 1989) and other salmonids (Ferguson and Fleming 1983; Beacham et al. 1989; Waples and Teel 1990). Higher levels of temporal variation in allele frequencies have been observed in salmonids in some studies. However, these have been attributed to factors such as selective influences on individual loci (Dempson et al. 1988) or inadequate sampling of the population (Parkinson 1984). Hatchery populations of salmonids often display increased levels of temporal variation in allele frequencies relative to natural populations (Youngson et al. 1991; Waples and Teel 1990). The influence of escapes and introductions of farmed fish may be reflected in the relative temporal instability of allele frequencies in the River Polly.

The absence of high levels of temporal variation in allele frequencies suggests that the majority of populations examined were in equilibrium with respect to the forces of selection, drift, and gene flow and/or that these forces were not strong enough to produce large temporal fluctuations. Variation at the $_{m}$_MEP-2*_ locus appears to violate the usual assumption of selective neutrality for genetic protein variation (Verspoor and Jordan 1989; Jordan et al. 1990; Jordan and Youngson 1991). However, there was no evidence that the level of temporal variability was greater at $_{m}$_MEP-2*_ than at other loci. Therefore,

TABLE 5. Values of F_{ST} and χ^2 for allele frequency differences among year-class samples for single sample sites in Scotland (1987–89). The sample size (N) refers to the total number of individuals collected at each site.

Locus	F_{ST}	χ^2	P
R. Nith (3 year-classes, N = 150)			
PGM-2*	0.004	1.20	n.s.
$_{m}$MDH-3,4*	<0.001	<0.27	n.s.
IDDH-1*	0.015	4.47	n.s.
$_{s}$AAT-4*	<0.001	<0.27	n.s.
IDHP-3*	0.033	9.83	<0.01
$_{m}$MEP-2*	<0.001	<0.27	n.s.
Mean	0.011	3.28	n.s.
R. Creran (2 year-classes, N = 100)			
GPI-3*	<0.001	<0.18	n.s.
IDDH-1*	<0.001	<0.18	n.s.
$_{s}$AAT-4*	<0.001	<0.18	n.s.
IDHP-3*	<0.001	<0.18	n.s.
$_{m}$MEP-2*	<0.001	<0.18	n.s.
Mean	<0.001	<0.18	n.s.
R. Ewe (2 year-classes, N = 89)			
IDDH-1*	<0.001	<0.16	n.s.
$_{s}$AAT-4*	<0.001	<0.16	n.s.
IDHP-3*	0.103	18.33	<0.001
$_{m}$MEP-2*	<0.001	<0.16	n.s.
Mean	0.013	2.31	n.s.
R. Polly (3 year-classes, N = 142)			
GPI-3*	0.001	0.57	n.s.
$_{m}$MDH-3,4*	<0.001	<0.51	n.s.
IDDH-1*	0.025	7.10	<0.05
$_{s}$AAT-4*	0.020	11.36	<0.05
IDHP-3*	0.003	0.85	n.s.
$_{m}$MEP-2*	0.048	13.63	<0.01
Mean	0.029	16.44	<0.01
Manor Water (3 year-classes, N = 123)			
IDHP-2*	0.018	8.86	<0.05
$_{m}$MDH-3,4*	0.005	1.23	n.s.
IDDH-1*	<0.001	<0.22	n.s.
$_{s}$AAT-4*	<0.001	<0.44	n.s.
IDHP-3*	<0.001	<0.22	n.s.
$_{m}$MEP-2*	<0.001	<0.22	n.s.
Mean	0.001	0.25	n.s.
Upper R. Tweed (2 year-classes, N = 97)			
GPI-3*	0.006	0.16	n.s.
$_{m}$MDH-3,4*	0.011	2.13	n.s.
IDDH-1*	<0.001	<0.17	n.s.
$_{s}$AAT-4*	<0.001	<0.34	n.s.
IDHP-3*	<0.001	<0.17	n.s.
$_{m}$MEP-2*	<0.001	<0.17	n.s.
Mean	<0.001	<0.17	n.s.
R. Grimersta (3 year-classes, N = 148)			
IDDH-1*	0.013	3.74	n.s.
$_{s}$AAT-4*	0.032	12.43	<0.01
IDHP-3*	<0.001	<0.26	n.s.
$_{m}$MEP-2*	0.020	5.88	n.s.
Mean	0.019	5.58	n.s.
R. Thurso (3 year-classes, N = 147)			
PGM-1*	0.004	1.18	n.s.
IDDH-1*	<0.001	<0.26	n.s.
$_{s}$AAT-4*	0.001	0.58	n.s.
IDHP-3*	0.015	4.35	n.s.
$_{m}$MEP-2*	0.019	5.59	n.s.
Mean	0.009	2.64	n.s.
R. Spey (3 year-classes, N = 147)			
SOD*	0.004	1.18	n.s.
$_{m}$MDH-3,4*	<0.001	<0.27	n.s.
IDDH-1*	0.002	0.57	n.s.
$_{s}$AAT-4*	0.008	4.68	n.s.
IDHP-3*	<0.001	<0.27	n.s.
$_{m}$MEP-2*	<0.001	<0.27	n.s.
Mean	0.002	0.58	n.s.
R. Dee (3 year-classes, N = 141)			
ADH*	<0.001	<0.25	n.s.
GPI-2*	<0.001	<0.25	n.s.
$_{m}$MDH-3,4*	<0.001	<0.25	n.s.
IDDH-1*	<0.001	<0.25	n.s.
$_{s}$AAT-4*	0.009	5.08	n.s.
IDHP-3*	<0.001	<0.25	n.s.
$_{m}$MEP-2*	0.027	7.61	<0.05
Mean	0.010	2.82	n.s.
Leader Water (2 year-classes, N = 89)			
$_{m}$MDH-3,4*	0.034	6.05	<0.05
IDDH-1*	<0.001	<0.16	n.s.
$_{s}$AAT-4*	<0.001	<0.32	n.s.
IDHP-3*	<0.001	<0.16	n.s.
$_{m}$MEP-2*	<0.001	<0.16	n.s.
Mean	0.002	0.36	n.s.
Bowmont Water (2 year-classes, N = 99)			
IDHP-2*	<0.001	<0.18	n.s.
IDDH-1*	0.028	5.54	<0.05
$_{s}$AAT-4*	<0.001	<0.18	n.s.
IDHP-3*	<0.001	<0.18	n.s.
$_{m}$MEP-2*	<0.001	<0.18	n.s.
Mean	0.008	1.58	n.s.

TABLE 6. Hierarchical gene diversity analysis for all samples in Scotland (except those from River Polly).

	Absolute gene diversity		Relative gene diversity (%)			
Locus	Total	Within year-classes	Within year-classes	Among year-classes within tributaries	Among tributaries within rivers	Among rivers
IDHP-2*	0.01484	0.00973	65.6	1.1	19.6	13.7
$_m$MDH-3,4*	0.01196	0.01069	89.4	3.1	3.8	3.7
IDDH-1*	0.41585	0.40373	97.1	1.0	0.7	1.2
$_s$AAT-4*	0.31189	0.30627	98.2	0.7	0.5	0.6
IDHP-3*	0.17291	0.16127	93.3	2.7	0.7	3.3
$_m$MEP-2*	0.49191	0.47682	96.9	1.1	0.3	1.7
All loci	0.23656	0.22809	96.4	1.2	0.7	1.6

selective influences at the locus appeared not to be strong enough to produce temporal fluctuations in allele frequencies that were quantitatively different from those produced by stochastic processes. Despite this, the direction of allele frequency change at the locus might well be influenced by selective pressures, as found at the esterase-2 in Arctic char (*Salvelinus alpinus*) (Dempson et al. 1988).

A balance between gene flow and drift might be expected to prevent changes in allele frequencies over the long term for populations in equilibrium. However, the short-term fluctuations in gene frequencies that might still occur were not evident. This suggests that the effective sizes of the Atlantic salmon populations studied were generally large enough to prevent extreme levels of genetic drift. A contributing factor may be that effective population size is increased over that expected from adult numbers by the successful spawning of mature male parr (Hutchings and Myers 1988; Jordan 1990).

An indication of gene flow levels can be obtained by examining the spatial pattern of genetic variation among Atlantic salmon populations with low levels of genetic differentiation suggesting high levels of gene flow (Slatkin 1985). A major advantage of a hierarchical analysis of gene diversity is that the partitioning of genetic variation produces values for the levels among subpopulations that exclude the influence of temporal variation within subpopulations. This, in turn, allows the statistical significance of purely spatial heterogeneity to be estimated.

Significant genetic heterogeneity observed among putative Atlantic salmon subpopulations from different river systems within Scotland indicated that there was real population substructuring among river systems. However, this did not appear to be the case within the River Tweed system. The low level of genetic differentiation found in the River Tweed system appears to be exceptional, as significant genetic heterogeneity has been found within a number of other large river systems in Scotland and elsewhere (Ståhl 1987; Verspoor et al. 1991).

The amount of gene diversity accounted for by differences among sites in Scotland was only 2.3% of the total. In a study of similar scope, McElligott (1987) found an among-population gene diversity level of 4.3% in Ireland. Such low levels of genetic differentiation suggest that gene flow levels among populations are high (Slatkin 1985). Straying in the Atlantic salmon, although restricted by the innate homing behaviour observed in the species (Stabell 1984), must therefore occur at rates high enough to prevent extreme levels of genetic differentiation over the geographical range considered here. Comparison of these levels of genetic differentiation with that of the closely related brown trout over a similar geographical area reveals that Atlantic salmon populations are relatively genetically homogeneous (Fleming 1983; Stephen and McAndrew 1990).

Conclusion

The lack of temporal variation in allele frequencies observed within Scottish Atlantic salmon populations in this study suggests that effective population sizes are relatively large and/or that gene flow levels among populations are high. High levels of gene flow are also suggested by the relatively low levels of genetic differentiation found among populations. However, apart from $_m$MEP-2*, the loci considered here are assumed to be selectively neutral. Investigation of loci that are more strongly influenced by natural selection may reveal higher levels of differentiation and provide insights into other biological factors that shape the structure of Atlantic salmon populations.

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Linkage between Islands and Benthos in the Ohio River, with Implications for Riverine Management

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Thorp, J. H. 1992. Linkage between islands and benthos in the Ohio River, with implications for riverine management. Can. J. Fish. Aquat. Sci. 49: 1873–1882.

Anthropogenic reductions in braiding, meandering, and snag abundance have diminished habitat heterogeneity of regulated rivers, factors directly influencing island formation, retentive capacity of the ecosystem, and community diversity. Habitat heterogeneity associated with riverine islands should, therefore, be of paramount importance to the ecosystem and may require special management protection. To understand the influence of these alluvial formations on riverine benthos, macroinvertebrate assemblages were sampled near three islands in the Ohio River above Louisville, Kentucky, USA. Benthos was collected along six bank-to-bank transects located 1 km above and below islands and near the head, middle, and foot of islands. Islands have significant positive effects on invertebrate density and diversity that appear related to changes in physical habitat characteristics. Current velocity and substrate particle size are diminished in narrow channels between islands and shore, and areal extent of the littoral zone is enhanced within an otherwise deepwater region. Shallow water and slower currents promote growth of submerged vascular plants and macrophytic algae. Because of a relatively low exploitation by humans, islands probably enhance snag formation and input of organic matter, both factors having positive effects on macrofauna. Creation of selected riverine preserves near islands as a management tactic is recommended.

Les réductions d'origine anthropique de la réticulation, de la formation de méandres et de la fréquence des croches ont diminué l'hétérogénéité de l'habitat dans les cours d'eau aménagés, facteurs qui ont une influence directe sur la formation des îles, la capacité de rétention de l'écosystème et la diversité de la communauté. L'hétérogénéité de l'habitat associée aux îles fluviales devrait donc avoir une importance primordiale pour l'écosystème et peut exiger un régime spécial de protection. Pour comprendre l'influence de ces formations alluviales sur le benthos de la zone proche des berges, on a échantillonné des assemblages de macroinvertébrés près de trois îles se trouvant dans le fleuve Ohio en amont de Louisville (Kentucky), aux États-Unis. Le benthos a été recueilli le long de six transects de rive à rive situés à un 1 km au-dessus et au-dessous des îles et près de la tête, du milieu et de la queue des îles. Les îles ont des effets positifs notables sur la densité et la diversité des invertébrés, effets qui semblent liés à des modifications dans les caractéristiques physiques de l'habitat. La vitesse du courant et la granulométrie du substrat diminuent dans les chenaux étroits entre les îles et la berge, et la superficie de la zone littorale augmente dans une région d'eau profonde par ailleurs. la faible profondeur de l'eau et le ralentissement des courants favorisent la croissance des plantes vasculaires immergées et des algues macrophytes. Du fait qu'elles sont soumises à une exploitation relativement faible par les humains, les îles favorisent probablement la formation de croches et l'apport de matières organiques, deux facteurs qui ont des effets positifs sur la macrofaune. Il est recommandé comme méthode de gestion de créer des réserves protégeant des zones littorales choisies près des îles.

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Long, relatively pristine, and unregulated reaches of very large rivers have nearly vanished from developed nations (exceptions in North America include some rivers in Canada and Alaska) and are rapidly disappearing from the less-developed countries of our biosphere as a result of intentional and extensive alterations of natural rivers over the last 100 yr (Benke 1990). Prior to anthropogenic efforts to manage rivers by channelization and impoundment, medium to large rivers (orders 6–9) were highly braided with complex, anastomosing channels that significantly expanded opportunities for direct riparian influence (Cummins et al. 1984). In some ways, the rivers of 100 yr ago were more akin to modern, midorder streams than to today's large regulated rivers. Managed rivers are less shaded, deeper, less turbid, and more homogeneous in habitat than unregulated rivers; they also contain fewer debris dams and have a different mixture of allochthonous and autochthonous carbon sources (cf. Cummins et al. 1984; Ward and Stanford 1989). A reduction in river meandering and channel complexity (including fewer islands) probably had dramatic effects on ecosystem properties that, in combination with anthropogenic pollution, depressed the density and diversity of riverine communities.

The very large rivers of the world will probably remain regulated for the foreseeable future because population pressures and the need for hydroelectric power and commercial navigation are unlikely to abate. Unfortunately, most of the world's large rivers have scarcely been examined from an ecological perspective (Statzner and Higler 1985; Williams 1988; Haag and Thorp 1991). Lotic research has focused on either headwater streams or a few unregulated tropical rivers, and, indeed, only 4% of the publications on running waters have dealt with large rivers (Hynes 1989). In recent years, it has become clear that ecological predictions made by extrapolating knowledge from midorder and headwater streams have failed to explain adequately ecosystem behavior of very large rivers, especially those with extensive floodplains (Sedell et al. 1989).

Attempts to protect and partially restore large rivers must be based on sound ecological knowledge. One promising approach is to designate critical areas of a river for special protection while attempts are made to ameliorate conditions throughout the ecosystem. Habitats that seem to fit within this goal are riverine islands. Although few attempts have been made to study islands, the sparse data suggest that they enhance the biotic community (Gosse 1963).

The purposes of the present study were to investigate the influence of three islands in the Ohio River on the density, diversity, and species composition of the benthic macroinvertebrate assemblage, an important food web component of the river, and to examine these data in light of the potential need to establish critical habitat preserves. This research was part of an ongoing, collaborative study of the importance of temporally fluctuating combinations of physical, chemical, and biological factors in regulating riverine communities. I examined the effects of an island's location within the Ohio River (distance from a downstream dam), the direct influence of islands on benthos, and the relationships between benthic community characteristics and the interrelated physical parameters of water velocity, depth, and substrate type. As described later, these data can be applied to models for the restoration and management of rivers. This study was also meant to contribute to our general knowledge of ecosystem behavior of very large rivers.

Methods and Habitat Description

Study Sites

Based on average yearly discharge, the Ohio River is the second largest river in the United States (8127 m^3/s) surpassed only by the Mississippi below its confluence with the Ohio (U.S. Geological Survey Water-Data Report for 1989, based on 61 yr of data). The average annual discharge of the Ohio at Louisville is 3273 m^3/s (115 500 ft^3/s), with a historical range of 60 to 31 455 m^3/s. Peak current velocities during the sample period in late summer 1989 were approximately 0.7 m/s at the water surface in midchannel and 0.10 ± 0.05 m/s near the bank. The Ohio has a constricted channel upstream from Louisville but becomes a floodplain river downstream; however, small floodplains are common within portions of the constricted region. The river bottom is generally muddy or sandy nearshore but becomes sandy and gravelly in deeper water, as is usually the case in large rivers (Hynes 1970, p. 25); large patches of limestone cobbles and boulders occur frequently on either bank.

Benthic samples were collected near Six, Twelve, and Eighteen Mile Islands within the McAlpine Pool (Fig. 1) just upstream of Louisville (38°16′N, 85°47′W). These three islands are roughly similar in size (30–50 ha) and shape but differ in distance from both the mainland shore (which influences bottom characteristics of the channels) and the downstream dam at Louisville (which affects current velocity and, thereby, the size of suspended particles). Each island was apparently named for its approximate distance upstream from McAlpine Dam, the low head, navigation dam at Louisville. Six Mile Island is situated about 75 m from the Indiana shore; Twelve Mile Island is in the middle of the river (~300 and 500 m from Indiana and Kentucky shores, respectively); and the "chute" (a narrow channel between an island and the mainland) is less than 50 m wide between Eighteen Mile Island and the Kentucky bank (Fig. 1).

These three islands have similar origins and extant riparian and submerged vegetation. Silver maple (*Acer saccharinum*) and cottonwood (*Populus deltoides*) are the most abundant riparian trees, but sycamore (*Platanus occidentalis*), box elder (*Acer negundo*), black willow (*Salix nigra*), and various other species are also present. Shoal areas near islands and river banks support seasonal growth of benthic filamentous algae, pondweed (*Potamogeton* spp.), and eel or ribbon grass (*Vallisneria americana*). Each island is entirely of alluvial origin and contains silt loam and fine sandy loam soils (Kentucky State Nature Preserves Commission, unpubl. rep.). These islands can only be reached by boat, with most human activity occurring during summer weekends; however, tow barges regularly pass near all three islands.

Sampling Design and Biological Measurements

I collected benthic grab samples from the following six cross-river transects around each island (Fig. 1): "A," 1 km above the head of the island (presumably upstream from island effects); "B" and "E," across the head and foot of the island, respectively, at a minimum depth of 1 m; "C" and "D," one third and two thirds of the distance downstream from the head, respectively; and "F", 1 km below the island. Six grab samples were retrieved along each transect (Fig. 1) for a total of 108 samples in the study (3 islands × 6 transects/island × 6 samples/transect).

Benthic samples were collected with a Ponar grab from substrates composed primarily of either mud, sand, or gravel (small to large). Shoreline areas composed of either wood snags or cobbles and boulders were avoided, as these bottom types could not be sampled effectively. Although this eliminated substrates with distinctive invertebrate assemblages (cf. Thorp et al. 1985), systematic error was reduced among sites by maintaining sampling consistency. (Species composition and secondary production of fauna on these substrates are being determined in a separate study (M. D. Delong and J. H. Thorp, unpubl. data).) Invertebrates were collected from all transects over a 3-wk span in late September and early October of 1989 when water temperatures were near 22°C from surface to bottom (usually ≤1°C difference between channel and bank).

Grab samples were sieved in the field through a 0.5-mm-mesh (U.S. Geological Series No. 35) and preserved in 70% EtOH + Phloxine B vital stain. Invertebrates were sorted in the laboratory and identified using keys in Merritt and Cummins (1984) and Thorp and Covich (1991). Body lengths (anterior–posterior) of Asiatic clams (*Corbicula fluminea*) were measured to provide information on size distribution of a numerically dominant species.

Habitat Characterization

Differences in water velocities among sample sites were measured 1 m below the water surface with a Marsh–McBirney velocity meter (model 201-D). Although very accurate for most of the water column, this meter cannot gauge velocities at the substrate surface where most riverine benthos reside. Therefore, current velocity data were used for macrocomparisons among shallow and deepwater sample locations rather than as a measure of microhabitat differences.

Predominant substrate types were estimated by wet sieving and qualitative field observations. Five of the seven substrate categories employed generally corresponded to those listed in Hynes (1970, p. 24): SI (silt and clay), FS (fine and very fine sand with some silt), CS (coarse sand), GR (gravel), and PB

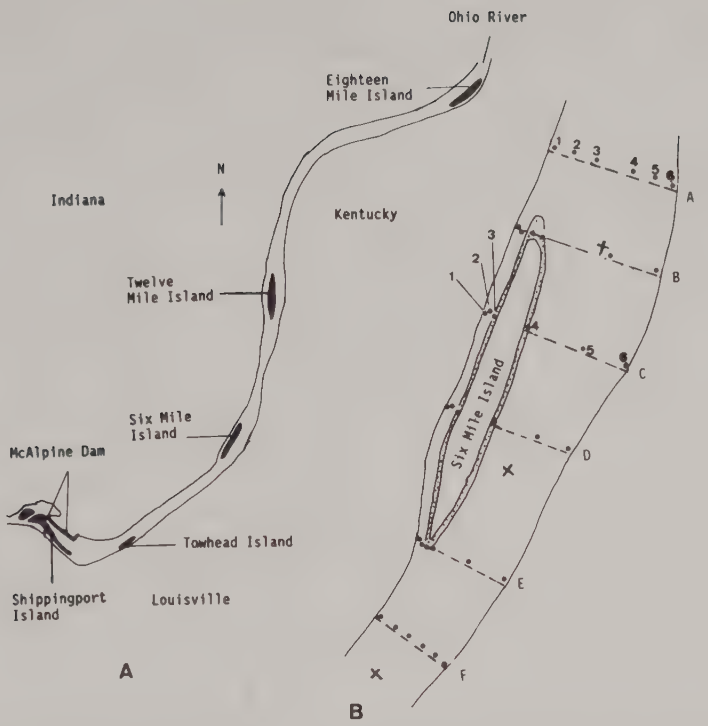

FIG. 1. (A) Stretch of the Ohio River as it flows southwesterly past Eighteen Mile Island through a navigation lock (between Shippingport Island and the Kentucky shore) or over and through the McAlpine Dam (an elongated z-shaped dam between Shippingport Island and the Falls of the Ohio near the Indiana shore); relative positions of the three islands studied here are shown. (B) Enlargement of the area around Six Mile Island showing the approximate positions of the six bank-to-bank transects (A–F), each with six sample sites (1–6); the crosses mark the midchannel area and are separated by 1.6 km (1 river mile); the narrow channel between the island and mainland is known as a chute.

(pebbles). Categories MG (muddy gravel) and SG (sandy gravel) were added because some habitats had markedly heterogeneous substrates during the sample period. Presence of vascular plants (mostly *Vallisneria*) or filamentous algae was recorded.

Statistical Analyses

Because macroinvertebrate abundances were low and spatial distributions of individual species were highly variable, the data were not normally distributed. Failure of traditional transformation techniques to normalize the data necessitated use of nonparametric techniques, principally Kruskal–Wallis ranked tests and Pearson's product moment correlations. Results were analyzed using subroutines of the Statistical Analysis System (SAS Institute, Inc. 1985); significance levels of 0.05 or lower were employed. Although invertebrates were usually identified to genus (Table 1), a priori statistical analyses were performed (unless otherwise noted) on 16 group variables: total invertebrate density, taxonomic richness, size of *Corbicula fluminea*, and densities of *Hydra*, Turbellaria, Oligochaeta, Hirudinea, Gastropoda, Bivalvia, Amphipoda, Ephemeroptera, Trichoptera, Chironomidae (larvae of all true midges), *Coelotanypus* (a predatory and overwhelmingly abundant midge), total "predatory" chironomids, and total "gatherer" midges.

Results and Discussion

The present study was restricted to analyses of responses of benthic macroinvertebrates to physical features associated with islands in one portion of a very large river. Ongoing research at the University of Louisville has been designed to evaluate the importance of competition, predation, and different sources of organic matter in shaping benthic and planktonic assemblages within the Ohio River.

Pool Effects

The construction of low head dams in the lower Ohio River over the last century altered this ecosystem into a series of flowing pools, averaging slightly over 100 river km in length. Differences in water velocity, river width, and substrate particle size are commonly observed from the shallower upstream section to the deeper downstream portion of a pool (J. H. Thorp, unpubl. data). Consequently, one might expect progressive changes in benthic and planktonic assemblages along the upper pool – lower pool gradient (cf. Delong and Payne 1985).

Contrary to our expectations, the relationship between longitudinal position within the lower McAlpine Pool and characteristics of the benthic fauna did not change in a consistent manner. Only five of the 16 independent, biotic variables tested were significantly affected by site within the pool, and their

TABLE 1. Mean abundance (no./m^2) of benthic taxa collected near three Ohio River islands in 1989.

	Six Mile Island	Twelve Mile Island	Eighteen Mile Island
Total invertebrate density	1076.1	633.2	859.4
Phylum Cnidaria (class Hydrozoa)			
Hydra vulgaris	22.4	8.0	3.7
Phylum Platyhelminthes			
Class Turbellaria	10.0	18.1	12.2
Phylum Nematoda	—[a]	—[a]	—[a]
Phylum Entoprocta			
Urnatella gracilis	—[a]	—[a]	—[a]
Phylum Bryozoa (Ectoprocta)	—[a]	—[a]	—[a]
Phylum Annelida			
Class Oligochaeta	731.5	279.6	312.6
Class Hirudinea (*Placobdella montifera*, *Placobdella* sp., and Glossiphoniidae)	4.8	1.6	2.1
Phylum Mollusca			
Class Gastropoda			
Amnicola spp.	1.1	0.5	3.2
Ferrissia spp.	2.1	1.1	2.1
Leptoxis praerosa	0	0	0.5
Lithasia obovata	0.5	0	1.1
Lithasia verrucosa	0	0.5	1.1
Pleurocera canaliculatum	5.4	2.1	2.7
Class Bivalvia			
Corbicula fluminea	175.9	169.6	216.4
Phylum Arthropoda			
Subphylum Crustacea (order Amphipoda)			
Gammarus fasciatus	22.8	12.6	21.3
Subphylum Chelicerata (class Arachnida)			
Class Arachnida (Acari)	0.5	0.5	1.1
Subphylum Uniramia (class Insecta)			
Order Ephemeroptera			
Caenis	10.7	3.7	3.7
Ephemeralla	0	0	0.5
Hexagenia	6.9	22.9	26.8
Stenacron	2.1	4.2	4.2
Stenonema	0	0	0.5
Order Odonata (Anisoptera) (*Neurocordulia* and *Somatochlora*)	0	0	1.1
Order Coleoptera			
Stenelmis	0.5	0	0
Order Trichoptera			
Cheumatopsyche	0	0	0.5
Hydropsyche	1.6	1.6	12.2
Hydroptila	95.7	0	0
Nectopsyche	0.5	0	0.5
Oecetis	1.6	2.7	8.5
Polycentropus	0	0.5	0
Order Diptera			
Family Ceratopogonidae			
Probezzia	0	0.5	1.6
Family Chaoboridae			
Chaoborus	1.1	0	0
Family Chironomidae			
Subfamily Tanypodinae			
Ablabesmyia[b]	0	0	1.6
Clinotanypus[b]	0	0	3.7
Coelotanypus[b]	64.9	72.3	117.5
Procladius[b]	2.7	1.6	0.5
Thienemannimyia[b]	2.7	1.1	1.6

TABLE 1. (*Concluded*)

	Six Mile Island	Twelve Mile Island	Eighteen Mile Island
Subfamily Chironominae			
Axarus	22.3	0	0
Cricotopus	22.8	1.6	6.4
Cryptochironomus[b]	6.4	9.0	17.0
Dicrotendipes	2.7	0.5	0
Nanocladius	0.5	0	4.2
Parachironomus[b]	0	0	0.7
Paracladopelma	0.5	0.5	0
Paratendipes	0.5	0.5	10.6
Phaenopsectra	0	2.7	0
Polypedilum	20.3	7.4	59.0
Rheotanytarsus	5.3	0	0.5
Stenochironomus	0.5	1.1	0
Tanytarsus	1.1	0	0

[a] Taxon present but not enumerated.

[b] True midge that is principally a predator; all other midges primarily obtain food in some other fashion, most as "gatherers."

mean values did not vary in a progressive fashion along the downstream gradient (comparisons among islands with data pooled across transects and samples). Average taxonomic richness and total invertebrate densities were significantly different among islands ($\chi^2 = 5.91$ and 6.86, respectively, $P < 0.05$), with the greatest means at the lower island and smallest values at the intermediate island. Total chironomids and the gatherer functional group of midges were most numerous near the upstream island and least dense at the middle island ($\chi^2 = 8.33$ and 10.74, $P < 0.05$ and 0.01, respectively); similarly, Asiatic clams were largest near Eighteen Mile Island and smallest near Twelve Mile Island ($\chi^2 = 9.09$, $P < 0.01$). In general, the intermediate island contained fewer invertebrate species and numbers than either the upstream or downstream island. A likely explanation for the poor fit of these data to a longitudinal gradient model within the McAlpine Pool is that all islands in the McAlpine Pool are in the lower portion of the pool; as a result, the "pool effect" was hidden within a more important, site-specific "island effect."

Island Effects

Islands interrupt the deepwater regions of this ecosystem by providing shallow-water habitats and access to different forms of food. Like areas along mainland banks, these littoral areas foster autochthonous production and furnish an alternative source of organic matter to the seston transported from floodplains and tributary streams. Macrophytes growing near island and mainland shores also afford greater habitat heterogeneity for benthic species. By adding a riparian zone in the "middle" of a river, islands serve as an additional source of allochthonous, partially labile, organic matter from recently fallen leaves (cf. Cummins and Klug 1979; Cummins et al. 1989). Terrestrial animals falling into the water from riparian plants and aquatic organisms growing on fallen trees are also considered a major source of high-quality food in rivers (Benke et al. 1984). Furthermore, shade from these trees and slower currents around islands alter light penetration and water temperatures.

The littoral zone benthos around islands appears similar to that present in shallow-water areas of the mainland bank (based on analyses of samples 1 and 6 versus 3 and 4 for transects B–E). In fact, none of the 16 independent variables tested showed significant variability between island and mainland littoral habitats (Kruskal–Wallis ranked test: $P > 0.05$ in all cases). When analyzed by individual island, only oligochaete density and total invertebrate density were significantly greater near the mainland shore (for Six Mile Island only), and the latter variable probably reflects to a major degree changes in the abundance of worms ($\chi^2 = 6.89$ and 7.46, respectively, $P < 0.01$). Estimated mean oligochaete densities were 475.3 and 803.1 worms/m^2 for island and mainland littoral areas, respectively; total invertebrate densities averaged 908.4 and 1199.4 individuals/m^2 for island and mainland shore habitats, respectively. Therefore, for the three islands studied here, the littoral benthos of island banks are generally equivalent to those on or in particulate sediments of mainland banks. An important caveat, however, is that these islands are entirely depositional in nature (composed principally of sand and finer particles) whereas the mainland shores contain some habitats with extensive beds of cobbles and boulders. Concurrent studies indicate that these latter habitats have an invertebrate fauna distinct from, and sometimes richer than, those found on particulate substrates near the three islands studied here (K. Greenwood and J. H. Thorp, unpubl. data on snail fauna on cobbles; M. D. Delong and J. H. Thorp, unpubl. data on secondary production on cobbles and snags).

To determine whether biotic effects of an island extend downstream below bordering, shallow-water regions, I compared benthos in transect A with that in transect F (~1 km above or below islands, respectively). Among the 16 group variables, only the gatherer functional feeding group of midges (hereafter called gatherer midges) was significantly different for pooled island data ($\bar{X} = 11.7$ versus 189.3 midges/m^2 for sites above and below islands, respectively; $\chi^2 = 3.99$, $P < 0.05$). When analyzed by island, results differed in only minor and inconsistent ways. *Corbicula* density ($\bar{X} = 226.4$ versus 32.0 clams/m^2) and size ($\bar{X} = 16.38$ versus 5.21 mm) were elevated upstream of Twelve Mile Island ($\chi^2 = 4.43$, and 4.35, respectively, $P < 0.05$). In contrast, at Eighteen Mile Island the three variables which were significantly different had greater mean values downstream of the island (total invertebrate density: $\bar{X} = 520.6$ versus 1253.7/m^2, $\chi^2 = 3.69$, $P < 0.05$; total chironomids: $\bar{X} = 80.4$ versus 444.0/m^2, $\chi^2 = 4.41$, $P < 0.05$; and gatherer midges: $\bar{X} = 19.1$ versus 386.6/m^2, $\chi^2 = 3.97$, $P < 0.05$). Hence, results suggest that the potential influence of islands on benthos does not extend much beyond the immediate shallow borders in a downstream direction, at least for the parameters I measured.

Finally, I compared diversity and density of benthos in the narrower and more shallow chute (sample 2 or 5, depending on the island, for transects B–E) with that in the wider and deeper midchannel region (the opposing sample 5 or 2; Fig. 1) to determine whether islands influence riverine benthos located lateral to islands. For this measure, islands had a strong influence on benthos. In general, densities were greater and assemblages were more diverse within island chutes (Fig. 2). Of the statistically significant comparisons, all followed this pattern ($P < 0.05$ for total Chironomidae and Turbellaria and $P < 0.01$ for *Hydra*, total Oligochaeta, *Coelotanypus*, and total predatory midges). *Corbicula*, total caddisflies, and gatherer midges were the only groups somewhat more dense in midchannel regions, but all such comparisons were nonsignificant.

A definitive explanation for why the chute environment is more favorable to benthic invertebrates than the midchannel region is not possible at this point in our knowledge of rivers. However, current velocity and wave action tend to be lower in chutes, and the water is shallower (unpubl. data), factors which appear to promote greater densities and diversity within the community, as discussed below. Furthermore, the substrate particle size is typically smaller, and the sediment contains more organic matter (personal observations); based on previous studies of riverine benthos (e.g. Haag and Thorp 1991), these substrate differences may have the greatest direct impact on the nature of the benthos.

Influence of Water Depth, Current Velocity, and Substrate Type

Because water depth, current velocity, and substrate type have some causal linkage in rivers, it is not always possible to distinguish clearly the response of benthos to a single factor. Bearing that in mind, results of this study indicate that abundances of most benthic species in the Ohio River were negatively correlated with both water depth and current velocity and were strongly influenced by substrate particle size and presence or absence of macrophytes, as documented below.

Eleven of 16 groups examined showed a negative correlation with water depth (for pooled samples, transects, and islands), but only five were significant (i.e. total invertebrate density and abundance of Oligochaeta: $P < 0.0001$; and size of Asiatic clams, density of *Coelotanypus*, and total abundance of predatory midges: $P < 0.05$ for Pearson's product moment correlations). None of the dependent variables had significant positive relationships with water depth. These results for the Ohio River initially seem to contrast with those reported for the Tennessee River below Kentucky Lake lock and dam (Haag and Thorp 1991), where most species showed a positive relationship with water depth. However, nearshore habitats (and all depths <3.1 m) were not sampled in the Tennessee because of extensive fluctuations in water level. Such shallow habitats supported most of the species in the present study.

Twelve invertebrate groups were more abundant in slower water (based on Pearson's product moment correlations), and five of these were significantly so (i.e. total density, Oligochaeta, *Coelotanypus*, and total predatory midges: $P < 0.01$; and total chironomids: $P < 0.05$). The bivalve *Corbicula* also tended to be larger in slower water ($P < 0.05$); however, these Asiatic clams were the only invertebrates significantly more abundant in faster water ($P < 0.05$), possibly reflecting the fact that they depend more directly and heavily on suspended organic matter than do other invertebrate groups examined here. A direct influence of currents on benthos is probably less widespread and influential in general within large rivers than indirect effects of water movement on substrate particle size and plant abundance (see following discussion). However, water velocity by itself can bring about a shift in the composition of riverine benthos, as demonstrated by Beckett and Miller (1982) for Ohio River macroinvertebrates colonizing multiplate samplers.

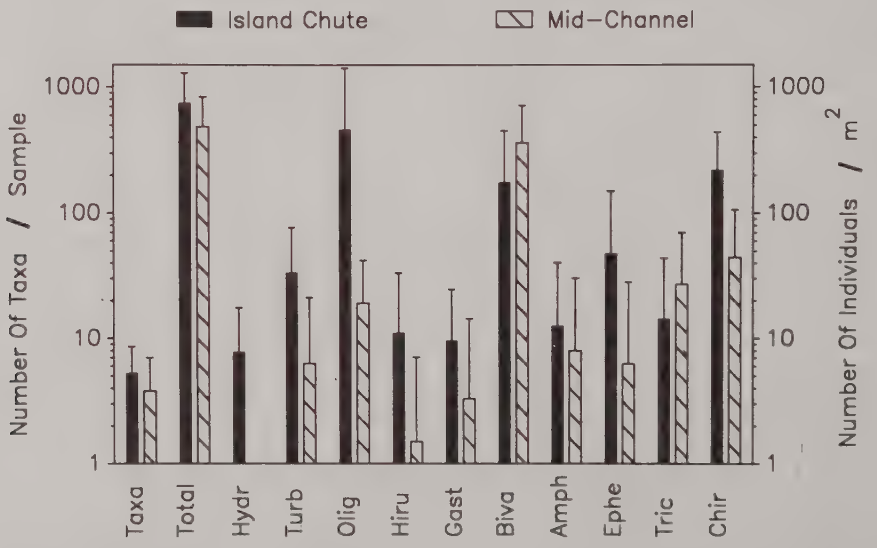

FIG. 2. Diversity and density of benthos in the narrower chute or wider midchannel region around three islands in the Ohio River. Values are means + 1 SD for taxonomic richness (Taxa, only dependent value on left ordinate scale), total invertebrate density, and densities of *Hydra* (Hydr), Turbellaria (Turb), Oligochaeta (Olig), Hirudinea (Hiru), Gastropoda (Gast), Bivalvia (Biva), Amphipoda (Amph), Ephemeroptera (Ephe), Trichoptera (Tric), and Chironomidae (Chir).

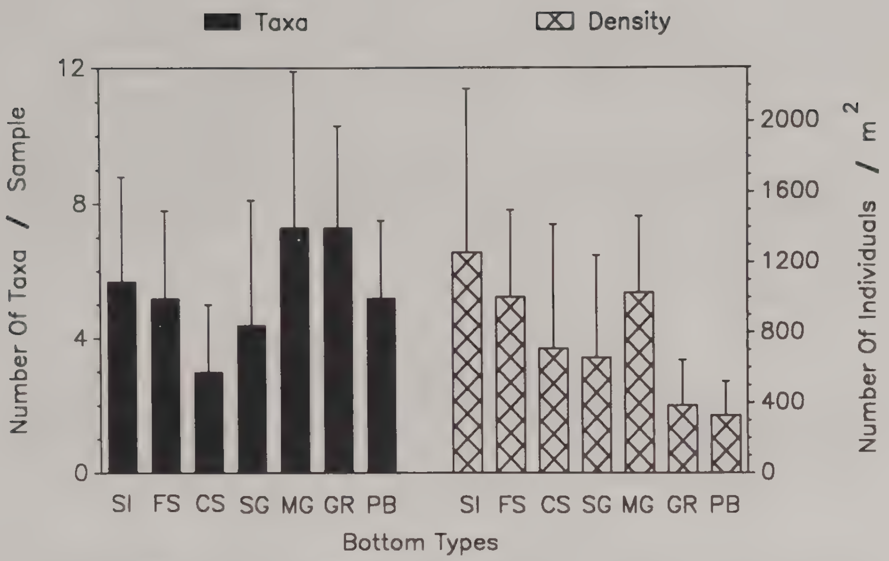

FIG. 3. Relationships between substrate size and both diversity (left ordinate scale) and total density (right ordinate scale) of benthos near three islands in the Ohio River. Values are means +1 SD for benthos on silt and clay (SI), fine and very fine sand (FS), coarse sand (CS), sandy gravel (SG), muddy gravel (MG), gravel (GR), and pebbles (PB).

Nearly 70% of the benthic grabs taken in this study were from sediments with small particle sizes (silt–sand) because the sampling emphasized island and mainland shores. This does not reflect the proportion of these substrates in the entire river, however, as shallow areas with wood snags, cobbles, or boulders were completely avoided and the largest portion of the river, the deepwater regions with typical pebble, gravel, and coarse sand bottoms, represented a minority of the samples.

Taxonomic richness and total invertebrate density were significantly influenced by substrate particle size ($\chi^2 = 20.31$ and 28.14, respectively, $P < 0.01$), a pattern previously shown for Tennessee River benthos (Haag and Thorp 1991). Diversity was greatest on gravel and muddy gravel and least on coarse sand in the Ohio River (Fig. 3). Total density was highest on silt–clay bottoms if the oligochaetes were included; otherwise, total abundance peaked on sandy gravel. Similar responses to particle size were evident for most invertebrate groups (Fig. 4). Coarse sand or pebbles supported the fewest individuals in 100% of the cases where density of a group was significantly related to sediment size (i.e. *Hydra*, Turbellaria, Oligochaeta, Amphipoda, Ephemeroptera, Trichoptera, total Chironomidae, *Coelotanypus*, and total predatory chironomids: $\chi^2 = 30.06$, 19.64, 40.15, 18.64, 26.20, 19.56, 27.02, 35.43, and 30.36, respectively, $P < 0.01$ in all cases). Coarse sand was the least colonized substrate for 60% of all groups tested, while midges and oligochaetes were seldom found on pebbles. The most favorable substrate was less consistent among groups, but 70% of those tested had highest densities on either muddy gravel or silt–clay bottoms. Other researchers have also confirmed the importance of substrate size to benthic assemblages (e.g. Thorup 1964; Resh and Lamberti 1979), with maximum densities often occurring on medium-sized particles (Rabeni and Minshall 1977; Williams and Mundie 1978).

Among all substrates, the dominant benthic groups within this portion of the Ohio River (Table 1) were oligochaetes (present in 90% of all samples), Asiatic clams (77% of samples), and chironomids (71%). The estimated average density of worms was 440 animals/m^2 (their density was difficult to gauge precisely because of worm fragmentation during sieving); they were especially abundant in muddy, shallow-water habitats. *Corbicula fluminea* was the most common species within the river (mean density of 187/m^2). Although biomass values were not recorded, this exotic species also appeared to dominate total invertebrate biomass, despite the fact that it was extremely rare to encounter individuals in the Ohio River which had reached even half their reported maximum body size (see Chap. 11 in Thorp and Covich 1991). Asiatic clams colonized all habitats but seemed to prefer, or survived best in, bottoms of coarse sand. Native unionid mussels are important in many areas of the Ohio River, but were not collected with a grab sampler in this study; however, while scuba diving, I have observed a few individuals just upstream of Eighteen Mile Island. Chironomids averaged 158 individuals/m^2 and occurred most frequently in substrates composed of a mixture of mud and either gravel or fine sand. In studies of the lower Mississippi River, midchannel sand habitats were dominated by chironomids (Beckett et al. 1983). *Coelotanypus* represented 54% of the midges collected in my study, and this predator was most dense on muddy gravel. Overall, 63.4% of the midges identified in this study are considered carnivorous, while 82% consume animals all or part of the time (Merritt and Cummins 1984).

Taxonomic richness and total invertebrate density were significantly higher when filamentous algae or vascular plants, such as *Vallisneria*, were present (Fig. 5) ($\chi^2 = 9.10$ and 10.28, respectively, $P < 0.01$). Similar patterns were evident when densities of individual taxa were compared in the presence or absence of plants (significant for Oligochaeta, Amphipoda, total Chironomidae, and gatherer midges: $\chi^2 = 9.92$, 7.38, 8.73, and 16.02, respectively, $P < 0.01$, and for Gastropoda, *Coelotanypus*, and total predatory midges: $\chi^2 = 3.87$, 4.73, and 3.91, respectively, $P < 0.05$). These results were expected for riverine benthos, based on studies in lentic eco-

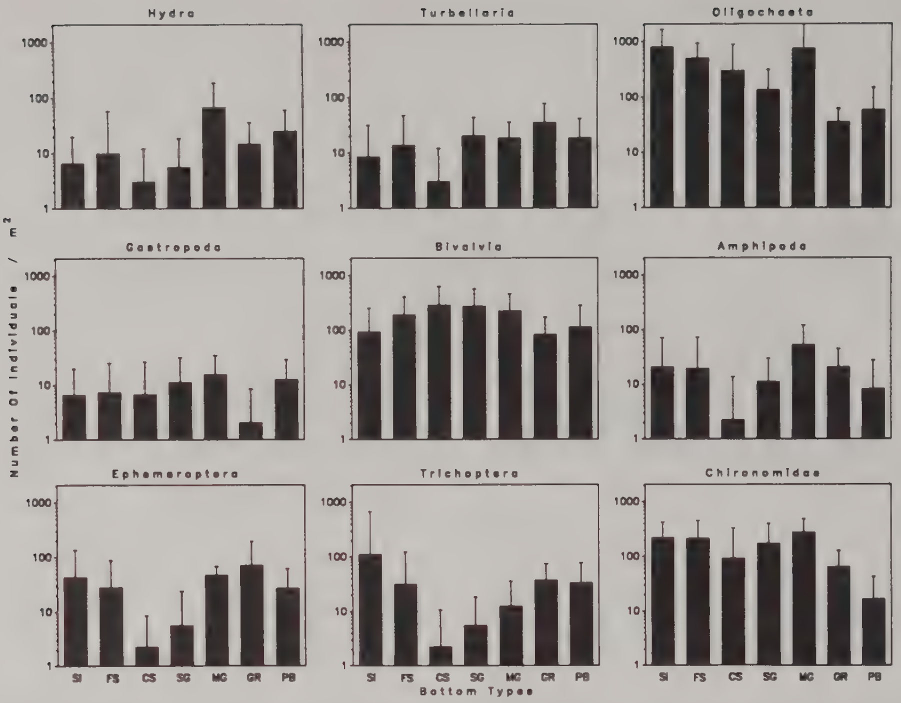

FIG. 4. Abundance of nine invertebrate groups on selected substrate types near three islands in the Ohio River. Values are means +1 SD for benthos on silt and clay (SI), fine and very fine sand (FS), coarse sand (CS), sandy gravel (SG), muddy gravel (MG), gravel (GR), and pebbles (PB).

systems (e.g. Hershey 1985; Thorp 1988) where macrophytes provide partial refuges for some invertebrates and may serve as additional sources of food.

Importance of Local Habitats in the Ecosystem Continuum Concept

Large rivers have not been examined in an ecosystem context as well as headwater streams and essentially represent ecological "black boxes." Current depictions of large river ecosystems are based primarily on (1) the river continuum concept (RCC: Vanotte et al. 1980) and subsequent modifications (e.g. Minshall et al. 1983, 1985), (2) the serial discontinuity concept (Ward and Standford 1983), which integrates effects of large dams and reservoirs on the RCC, and (3) the flood pulse concept in river–floodplain systems (Junk et al. 1989) and its relationship to the RCC (Sedell et al. 1989). Such notable theories are, unfortunately, primarily based on research conducted in medium-sized to small streams. By concentrating on demonstrably important downstream and lateral processes, these theories have, in my opinion, ignored important local phenomena which influence community structure and function.

An implicit assumption seems to exist (see fig. 1 in Vannote et al. 1980) that benthic communities of large rivers consist almost entirely of collectors (filterers and gatherers) and a few predators. In contrast, data from the present and concurrent studies (K. Greenwood and J. H. Thorp, unpubl. data; M. D. Delong and J. H. Thorp, unpubl. data) do not support this assumption. Although "collector" clams and mussels are important in some substrates of the Ohio River, different functional feeding groups, such as grazing snails and omnivorous amphipods, are prominent in other habitats (e.g. in patches of cobbles, wood snags, and macrophyte beds). The importance of various trophic guilds to the ecosystem cannot be evaluated with confidence until we possess comprehensive data on relative densities, biomasses, and secondary productivities; such data are almost entirely lacking. Results of the present study and ongoing research suggest, however, that riverine food webs are habitat based and more complex than originally assumed. For these and other reasons, environmental managers should integrate habitat-specific concerns into their management strategies, as discussed below.

Management of Critical Riverine Habitats

Flood control, hydroelectric, and navigation projects have greatly modified the ecological nature of almost all major rivers of the world (e.g. Cummins et al. 1984; Sedell and Froggatt 1984; Dister et al. 1990). For example, 90% of the original floodplains for the lower Mississippi River have been lost through construction of levees, a process that began more than 150 yr ago (Fremling et al. 1989). This has influenced sediment and organic input and has diminished the amount of habitat available for fish nurseries and invertebrate production. Recent interest in restoring productivity within some of these rivers has resulted in proposals for comprehensive management practices. Among the tools suggested by Petts et al. (1989) are flow modification, water quality control, and habitat management. Included in the last category is the construction of islands, a technique which has been employed in several large rivers, such as the Mississippi (Fremling et al. 1989), with consistently satisfactory results (Petts et al. 1989).

The three islands studied here had a significant positive influence on an important food-web component of the ecosystem, the benthic macroinvertebrate assemblage. Although density of species within McAlpine Pool was low on average and species

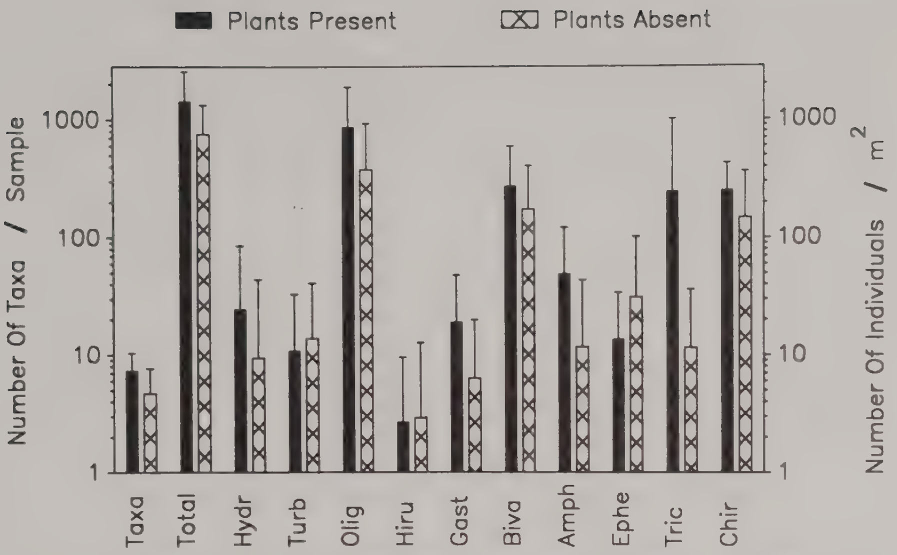

FIG. 5. Diversity and density of macroinvertebrates in the presence or absence of macrophytic algae and vascular plants around three islands in the Ohio River. Values are means + 1 SD for taxonomic richness (Taxa, only dependent value on left ordinate scale), total invertebrate density, and densities of *Hydra* (Hydr), Turbellaria (Turb), Oligochaeta (Olig), Hirudinea (Hiru), Gastropoda (Gast), Bivalvia (Biva), Amphipoda (Amph), Ephemeroptera (Ephe), Trichoptera (Tric), and Chironomidae (Chir).

distributions were often patchy (making it more difficult to detect statistically significant island effects), the presence of islands operated in several ways to enhance density and diversity of macrofauna. Littoral areas were present in otherwise deep water zones, and these shallow-water habitats supported more species than deepwater habitats. This was due in part to submerged macrophytes, whose presence in the littoral zone was consistently associated with a higher density and diversity of macrofauna. The riparian zones of islands near populated regions appear to produce more snags and leaf litter than comparable areas on nearby mainland banks because these ephemeral islands are usually less developed by humans. Studies have clearly shown that snag habitats are associated with higher secondary productivity in rivers (Benke et al. 1984; Sedell and Froggatt 1984), and more readily available, labile organic matter from abundant riparian vegetation should also boost production. Islands can decrease current velocity and substrate particle size in adjacent areas; within the narrow chute of the islands I examined, macroinvertebrates were significantly more abundant than in the broader, deeper, and faster flowing main channel.

Evidence from the present project strongly supports, therefore, the management hypothesis that islands are good for riverine invertebrates. From such data, one can infer that these alluvial islands also benefit fish populations, a conclusion strengthened by results of research on fish productivity near islands in the middle Zaire River (Gosse 1963, cited in Ward and Stanford 1989).

The lower Ohio River contains fewer than 40 islands over a stretch of 876 river km, which makes their importance to the river as a whole somewhat questionable at first glance. However, riverine islands and other slackwater areas may be even more valuable to the ecosystem because of their rarity. This makes protection of the few islands surviving navigation and flood control management practices a vital goal if we are to protect and restore river ecosystems. In conjunction with a comprehensive and much larger management plan suggested by others (e.g. Petts et al. 1989), I believe that protected zones should be established around carefully chosen islands in major rivers of the United States. These zones should be designed to safeguard not only the island itself (as is being done for many islands in the upper Ohio River in response to federal legislation and for Six Mile Island in the McAlpine Pool by the Kentucky State Nature Preserves Commission) but also some adjacent waters. Such preserves should limit human access to the islands, boat traffic in adjacent chutes, commercial harvesting of mussels, and gravel/sand removal operations immediately upstream of an island. Restoration measures could then be undertaken to foster habitat heterogeneity in these wildlife preserves. Furthermore, the U.S. Army Corps of Engineers in the United States and similar agencies in other countries should be encouraged to build islands and other habitat enhancements, where economically feasible, as part of river dredging operations.

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Assessment of the Impact of the Myxosporean Parasite *Ceratomyxa shasta* on Survival of Seaward Migrating Juvenile Chinook Salmon, *Oncorhynchus tshawytscha*, from the Fraser River, British Columbia

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Approximately 3.3% of more than 3500 seaward migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*) collected from the lower reaches and off the mouth of the Fraser River, British Columbia, between March and August 1985–87 were infected with *Ceratomyxa shasta* (Protozoa: Myxosporea). The fish were held live for up to 151 d before examination to allow the infections to become patent. The first infected fish were detected in samples taken in late May to early June, approximately 4 wk after the river water temperature had reached 10°C. By this time, 40–65% of the fish had been collected, indicating that the majority of the juvenile chinook salmon had left the Fraser River before the infective stage of the parasite was present. Significant differences in prevalence of *C. shasta* were associated with both the migration route chosen by the fish and their age. Fish that used the lower flow rate North Arm had a greater prevalence (6.8%) of infection than those that migrated down the Main Arm (2.1%). Age 0 fish had a significantly higher prevalence (5.2%) of *C. shasta* than the age 1 group (1.5%). It is concluded that *C. shasta* is not a major cause of mortality of downstream migrating juvenile Fraser River chinook salmon.

Environ 3,3% des jeunes saumons quinnats (*Oncorhynchus tshawytscha*), au nombre de plus de 3 500, recueillis pendant leur migration vers la mer dans le cours inférieur et devant l'embouchure du Fraser (Colombie-Britannique) entre mars et août, de 1985 à 1987, étaient infestés de *Ceratomyxa shasta* (Protozoa : Myxosporea). Les poissons ont été maintenus en vie pendant une période allant jusqu'à 151 jours avant l'examen, ce qui a permis aux infestations de se manifester. Les premiers poissons infestés ont été repérés dans des échantillons pris entre la fin mai et le début juin, environ quatre semaines après que la température de l'eau avait atteint 10°C. À ce moment-là, 40–65% des poissons avaient été capturés, ce qui indique que la majorité des jeunes quinnats avaient quitté le Fraser avant la phase infestante du parasite. Des différences significatives dans la prévalence de *C. shasta* étaient associées à la fois à la voie migratoire choisie par les poissons et à leur âge. Les poissons qui passaient par le bras nord, dont le débit est plus faible, présentaient une plus forte prévalence de l'infestation (6,8 %) que ceux qui passaient par le bras principal (2,1 %). Les poissons d'âge 0 présentaient une prévalence significativement plus élevée (5,2 %) de *C. shasta* que le groupe d'âge 1 (1,5 %). On en conclut que *C. shasta* n'est pas une des principales causes de mortalité des jeunes saumons quinnats du Fraser pendant leur avalaison.

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Ceratomyxosis, caused by the histozoic myxosporean *Ceratomyxa shasta* Noble, 1950, was first recognized as a disease of hatchery-reared salmonids in northern California (Noble 1950; Wales and Wolf 1955) and has been reviewed recently by Bartholomew et al. (1989a). The occurrence and pathogenicity of *C. shasta* in both wild and cultured salmonids in the Columbia River basin of Washington, Oregon and Idaho have been extensively documented (Rucker et al. 1954; Conrad and Decew 1966; Sanders et al. 1970; Udey et al. 1975; Zinn et al. 1977; Wood 1979; Johnson 1980; Ratliff 1981; Chapman 1986; Hemmingsen et al. 1986; Hoffmaster et al. 1988). Initially, the infection appears in the walls of the posterior region of the intestine, from where it spreads to other tissues and organs, including the liver, kidney, spleen, and somatic musculature (Bartholomew et al. 1989b).

In British Columbia, the occurrence of ceratomyxosis was first recorded in 1975 from juvenile chum salmon (*Oncorhynchus keta*) caught in the Strait of Georgia (Margolis and Evelyn 1975). Later, *C. shasta* was found to be widespread among stocks of wild prespawning salmonids, including chinook salmon (*Oncorhynchus tshawytscha*), in the Fraser River system (Arai and Mudry 1983; McDonald 1983; Bell and Traxler 1985), and Bower (1985) reported infections in migrating juvenile chinook salmon caught in the Fraser River near its mouth.

High mortalities due to *C. shasta* infection among juvenile chinook salmon exposed either directly by cage confinement in the lower Fraser River or in the laboratory by exposure to Fraser River water were reported by Ching (1984), Ching and Munday (1984a, 1984b), and Ching and Parker (1989), with a seasonal onset of the infection in late April (Ching and Munday 1984a). The results of these experiments led to speculation that ceratomyxosis may be a cause of high mortalities and a major contributor to the decline of Fraser River chinook stocks. To determine the prevalence of *C. shasta* and assess the impact of this pathogen on the survival of seaward migrating juvenile Fraser River chinook salmon, which comprise both wild and hatchery-

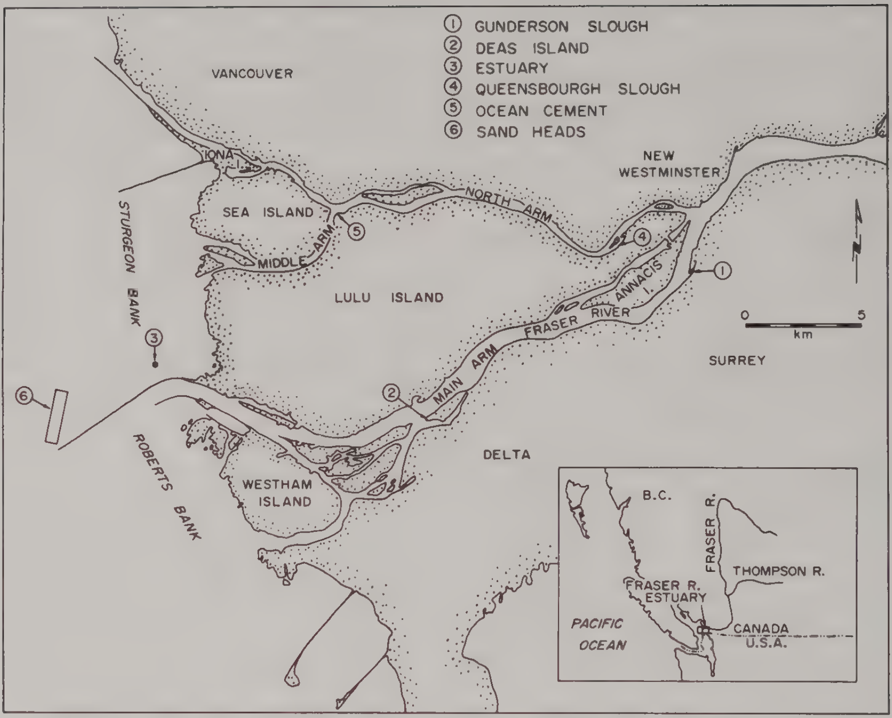

FIG. 1. Main Arm, North Arm, and mouth of the Fraser River, British Columbia, showing sampling stations for seaward migrating juvenile chinook salmon, 1985–87.

reared fish, weekly sampling was conducted in the lower river during the period of migration (March to August) in the years 1985–87.

Materials and Methods

During 1985–87, seaward migrating juvenile chinook salmon were captured live from the lower reaches of the Fraser River using a 15.5-m-long beach seine of 6.5-mm stretched mesh size operated from a 5.5-m aluminum riverboat. Sampling was conducted at four stations on the river (two on the North Arm and two on the Main Arm) between New Westminster and the river mouth (Fig. 1). (These stations are downstream from the locality used by Ching and Munday (1984a, 1984b) for their exposure experiments.) Additional samples were collected from the Fraser River estuary (station 3 in Fig. 1) by beach seining in 1985 and off Sand Heads (station 6 in Fig. 1) in 1986 and 1987 by two-boat trawling with a 3.3-m-square × 10-m-long beam trawl ending in a modified live trap bucket. The bucket was emptied every 5–10 min during the course of trawling operations to ensure that captured fish remained in good condition. In 1985, samples were collected from stations 1, 2, and 3 or stations 1, 4, and 5 (Fig. 1) in alternate weeks. In 1986 and 1987, fish were collected weekly from stations 1 and 4 and in alternate weeks from either station 2 or 5. Station 6 was sampled monthly. River surface water temperature was recorded during each sampling operation.

The chinook samples collected at each station were held separately in 189.5-L containers of Fraser River water and transported to and placed in holding facilities at the Pacific Biological Station, Nanaimo, within 8 h after capture. The containers were aerated using oxygen or battery-operated mechanical pumps with air stones; during warm weather, bagged ice was added as required to prevent the water temperature from reaching lethal levels. At Nanaimo, each fish sample was transferred to a separate 94.5-L circular tank containing running dechlorinated domestic fresh water, which was gradually replaced with running sea water (28–32‰ salinity) over a period of 48 h. In 1986 and 1987, all collected fish were vaccinated against vibriosis.

The collections were held between March and November, each for up to 151 d in ambient temperature sea water. In 1985 the mean ambient temperature, during the holding period, was 11.6°C and ranged between 10 and 16°C. In 1986 the mean ambient temperature was 11.4°C and the range was 9–17°C, and in 1987 the mean was 12.6°C with a range of 10–19°C. The fish were checked and fed a pelleted diet daily. All fish that died during the holding period were examined microscopically for the presence of *C. shasta* spores or pansporoblast stages. Between 40 and 50 microscope fields of fresh smears, comprising tissue scrapings and contents taken separately from the caeca, intestine, and gall bladder, were examined at 250–400× using a compound microscope equipped with phase contrast illumination. At the end of the holding period, all remaining fish from each sample were examined as described above.

Scale samples were taken from all fish collected in 1986 and 1987 for age determination.

Frequency data were compared using *G*-tests of independence. When the *G* values were marginally significant, they were adjusted with Williams' correction (Sokal and Rohlf 1981).

Results

Of more than 3500 juvenile chinook salmon collected from the lower Fraser River and off its estuary during the three years of the study, 3.3% were subsequently determined to be infected

TABLE 1. Overall prevalence (1985–87) of *C. shasta* infections among samples of seaward migrating juvenile chinook salmon from the Main and North arms of the lower Fraser River and off the mouth of the river.

	No. sampled (% of total sampled)	No. (%) infected
Main Arm	2129 (60.6)	45 (2.11)
North Arm	1050 (29.9)	71 (6.76)
Estuary and Sand Heads	335 (9.5)	0 (0.00)
Total	3514 (100.0)	116 (3.30)

with *C. shasta* (Table 1). Prevalences of infection differed significantly ($G = 40.04$; df = 1; $P < 0.001$) between the North Arm (sampling stations 4 and 5) and the Main Arm (sampling stations 1 and 2) of the river, 6.8% versus 2.1% for the three years combined (Table 1). No infections were found among the 335 fish collected in and outside the estuary.

Prevalences of *C. shasta* infection by sampling station and year ranged from 0% to more than 12% (Table 2). Annual prevalences of infection for all stations combined varied from 1.8% in 1985 to 4.7% in 1987 (Table 2). Among the chinook salmon caught in the lower river (stations 1, 2, 4, and 5) on and after the date of the first appearance of *C. shasta* in the samples, the prevalence of infection varied between 6.2% in 1985 and 8.7% in 1986 (Table 3).

Between 40% (1987) and 65% (1985) of the chinook salmon sampled annually from the river were caught prior to the collection date of the first infected fish. These annual percentages were inversely related to the date the river temperature reached 10°C. Infections of *C. shasta* were first detected among fish captured in late May or early June (Fig. 2), by which time the river temperature had warmed to 11–12.5°C. The earliest collection dates of infected fish were 3.5–4.5 wk after river temperatures reached 10°C. The year (1987) with the highest prevalence (4.7%) of *C. shasta* infection was also the year of earliest occurrence of 10°C water as well as the year of earliest occurrence (May 20) of infected fish.

Of the 2094 chinook salmon aged in 1986 and 1987, 77.1% were age 0 (Table 4). The proportion of age 0 and age 1 fish (both years combined) was similar in the North Arm and Main Arm samples ($G_{\text{adj}} = 2.66$; df = 1; $P < 0.5 > 0.1$), although in 1986, there was a difference ($G = 14.40$; df = 1; $P < 0.001$) in age composition between arms. However, the age composition (both arms combined) differed somewhat between years ($G_{\text{adj}} = 5.11$; df = 1; $P < 0.025 > 0.01$). The period of migration, as suggested by the age composition of the samples taken in 1986 and 1987 (Fig. 3), is more prolonged for age 0 than for age 1 fish.

The proportion of infected fish was higher among age 0 (5.2%) than age 1 fish (1.5%) for all fish aged in 1986 and 1987 (Table 4). This difference in infection prevalence according to age was more pronounced among North Arm than Main Arm samples. Of all infected fish, 92.3% were age 0 (Table 4) whereas only 77.1% of all fish sampled in 1986 and 1987 were age 0. Among the fish collected on and after the date of first detection of infection, 9.1% of the age 0 group were infected compared with 3.3% for the age 1 fish, although no age-associated differences were observed in 1986 presumably because of the small sample of age 1 fish (Table 5).

Discussion

The low overall prevalences of *C. shasta* infection during each of the three years of our study do not support earlier suggestions (Ching and Munday 1984a) that ceratomyxosis may be a significant cause of mortality of Fraser River chinook salmon. The impact of *C. shasta*, however, was greater in the latter one third to one half of the downstream run than in the earlier part of the run.

The earlier studies that yielded high mortalities (Ching 1984; Ching and Munday 1984a, 1984b; Bower and Margolis 1985; Ching and Parker 1989) used unnatural exposures to *C. shasta* either through cage confinement in the river or holding fish in water collected or pumped from the river. The differences between the results of the experimental investigations and the present study may be explained by one or more of the following circumstances: (1) the cages were placed in quiet areas of the river where the concentration of the infective stage of the parasite was likely greater than in the faster flowing waters through which juvenile chinook naturally migrate; other exposure experiments used water collected or pumped from similar, quiet regions of the river; (2) the fish in the earlier studies may have been exposed continuously to the infective stage for extended periods of time (5–27 d) compared with the likely more fleeting encounters by the naturally migrating fish; (3) the stress of confinement may have lessened the resistance of experimental fish to the parasite; and (4) the experimental studies may have involved single chinook stocks that individually were more susceptible to the infection than the mixed stocks of the wild population. Regardless of the reasons for the differences, the significant fact is that the results obtained from experimental exposures of juvenile chinook salmon to Fraser River water are

TABLE 2. Prevalence of *C. shasta*-infected fish among migrating juvenile chinook salmon sampled at several localities in the lower Fraser River and off its mouth, 1985–87.

	1985		1986		1987	
Sample station[a]	No. sampled	No. (%) infected	No. sampled	No. (%) infected	No. sampled	No. (%) infected
1	489	12 (2.45)	338	5 (1.48)	591	25 (4.23)
2	336	0 (0.00)	131	0 (0.00)	244	3 (1.23)
3	215	0 (0.00)	—	—	—	—
4	8	1 (12.50)	154	6 (3.90)	453	26 (5.74)
5	131	8 (6.11)	108	13 (12.04)	196	17 (8.67)
6	—	—	103	0 (0.00)	17	0 (0.00)
Total	1179	21 (1.78)	834	24 (2.88)	1501	71 (4.73)

[a] 1 = Gunderson, 2 = Deas Island, 3 = estuary, 4 = Queensbourgh, 5 = Ocean Cement, 6 = Sand Heads.

TABLE 3. Prevalence of *C. shasta* among seaward migrating juvenile chinook salmon collected on and after the date of the first appearance of infected fish in samples from the lower Fraser River.

Year	No. sampled after first infection (% total no. sampled)	No. (%) infected
1985	337 (35.0)	21 (6.23)
1986	275 (38.0)	24 (8.73)
1987	896 (60.4)	71 (7.92)

not applicable to the assemblages of stocks of this species undergoing their natural seaward migration.

Differences observed in infection prevalences between juvenile chinook salmon migrating through the North Arm (6.8%) and those migrating through the Main Arm (2.1%) of the Fraser River may be a result of the lower flow rate in the North Arm. It has been suggested that production of infective stages of *C. shasta* is associated with quiet waters (Ratcliff 1981). The lower flow rate in the North Arm could allow accumulation of higher concentrations of the infective stage of *C. shasta*. The lower flow rate may also decrease the rate of downstream movement of chinook salmon through the North Arm, thus exposing them to the infective stage of *C. shasta* for a longer period than those that use the Main Arm.

Because the overall proportions of age 0 and age 1 fish were similar in samples from both arms of the Fraser River, there is no evidence for selection, by fish age, of one migration route over the other. Thus, age composition was not a factor influencing the difference in infection prevalences between the North and Main arms. Further, the age composition of the fish in our samples (77.1% age 0 and 22.9% age 1) is similar to the overall freshwater age composition of Fraser River chinook salmon (Fraser et al. 1982). Hence, the overall *C. shasta* prevalences

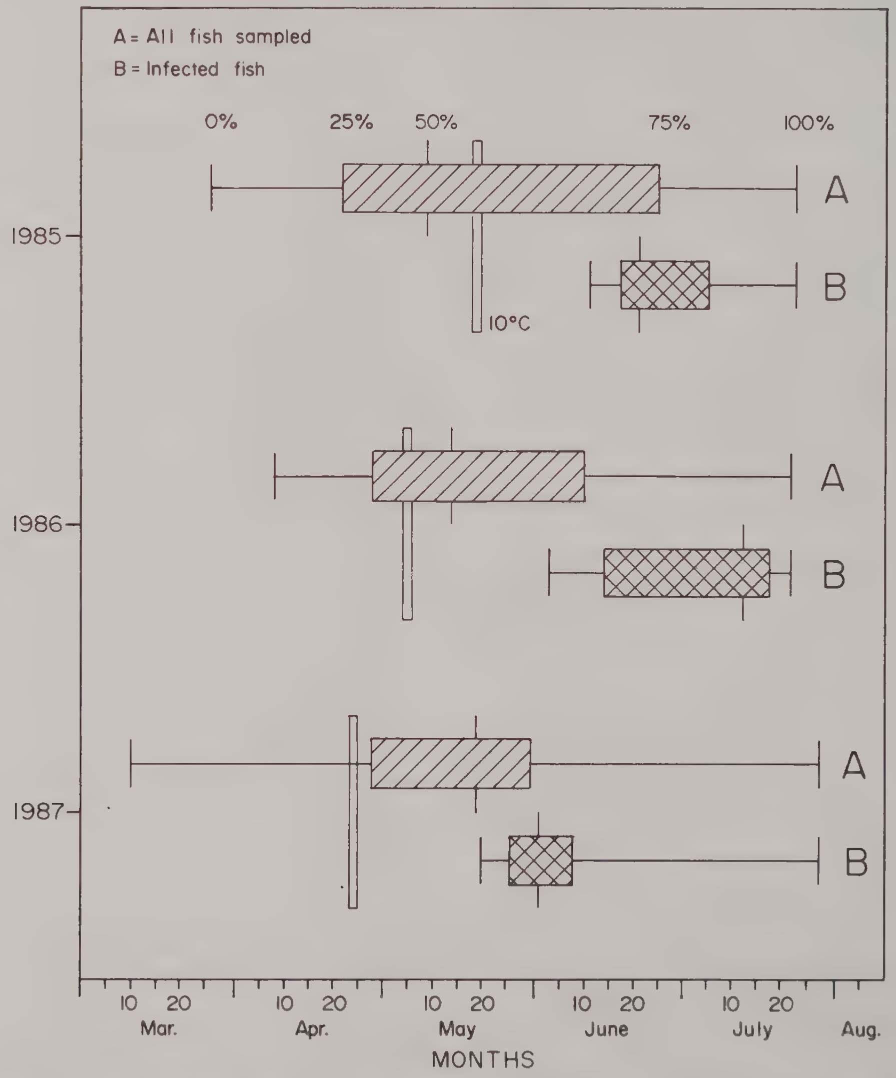

FIG. 2. Seasonal distribution of seaward migrating juvenile chinook salmon and of *C. shasta*-infected individuals, shown as a percentage of the total number of fish sampled and of the total number of infected fish, respectively, collected from the Main and North arms of the lower Fraser River, 1985–87, in relation to 10°C water temperatures. (Hatched areas represent the middle 50%.)

TABLE 4. Age composition of juvenile chinook salmon collected from the North Arm (NA) and the Main Arm (MA) of the Fraser River in 1986 and 1987 and of the *C. shasta*-infected fish in these samples.

	No. of fish aged			No. of infected fish				
					Age 0		Age 1	
	Total	Age 0 No. (%)	Age 1 No. (%)	Total	No. (% total no. infected)	% total no. of age 0 examined[a]	No. (% total no. infected)	% total no. of age 1 examined[b]
NA 1986	245	202 (82.4)	43 (17.6)	17	17 (100.0)	8.4	0 (0.0)	0.0
NA 1987	625	484 (77.4)	141 (22.6)	42	40 (95.2)	8.3	2 (4.8)	1.4
NA both years	870	686 (78.8)	184 (21.1)	59	57 (96.6)	8.3	2 (3.4)	1.1
MA 1986	446	310 (69.5)	136 (30.5)	5	4 (80.0)	1.3	1 (20.0)	0.7
MA 1987	778	618 (79.4)	160 (20.6)	27	23 (85.2)	3.7	4 (14.8)	2.5
MA both years	1224	928 (75.8)	296 (24.2)	32	27 (84.4)	2.9	5 (16.0)	1.7
Total 1986	691	512 (74.1)	179 (25.9)	22	21 (95.5)	4.1	1 (4.5)	0.6
Total 1987	1403	1102 (78.5)	301 (21.5)	69	63 (91.3)	5.7	6 (8.7)	2.0
Total both years	2094	1614 (77.1)	480 (22.9)	91	84 (92.3)	5.2	7 (7.7)	1.5

[a] % of no. in column 3.
[b] % of no. in column 4.

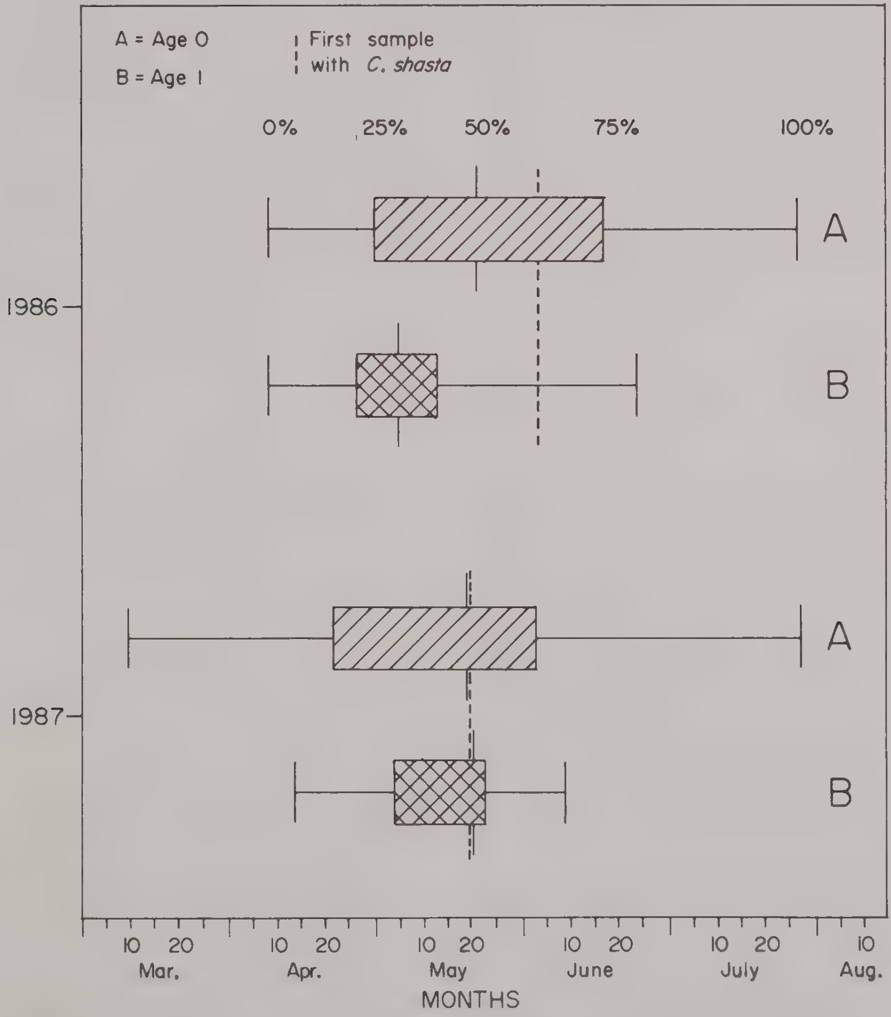

FIG. 3. Seasonal distribution of age 0 and age 1 seaward migrating juvenile chinook salmon, shown as a percentage of the total number of fish in the respective age group, collected from the Main and North arms of the lower Fraser River in 1985 and 1987. (Hatched areas represent the middle 50%.)

TABLE 5. Prevalence, by age class, of juvenile chinook salmon infected with *C. shasta* collected from the lower Fraser River in 1986 and 1987 on and after the dates the first infected fish were found. (Note: two infected fish in each year were not aged.)

	Age 0			Age 1		
Year	No. sampled	% total sampled	No. (%) infected	No. sampled	% total sampled	No. (%) infected
1986	257	50.2	21 (8.2)	12	6.7	1 (8.3)
1987	665	60.3	63 (9.5)	199	66.7	6 (3.0)
Total	922	57.1	84 (9.1)	211	44.0	7 (3.3)

determined in our study have not been biased by the age composition of our samples.

The higher prevalence of *C. shasta* observed in the age 0 group compared with the age 1 group was probably due to the longer migration period of age 0 fish. Chinook salmon of the former group continued to be caught into late July whereas few age 1 fish were present in the samples after mid-June. A greater proportion of the age 1 fish would, therefore, have left the river prior to the onset of the infectious period. It has been suggested (Ching and Munday 1984a, 1984b) that the infective stage increases in numbers as the season progresses, exposing the later migrating fish to a greater concentration of this stage. Possible behavioural differences between fish of the two age categories may have also influenced their prevalences of infection. For example, age 0 chinook salmon, because of their smaller size, may migrate more slowly through the infectious area of the river, thus increasing their exposure time to the infective stage of *C. shasta*; or they may have a greater tendency to hold in, or migrate through, areas of lower water velocity where higher concentrations of the infective stage may accumulate. It is also possible that the age 1 fish were from stocks that have a greater resistance to *C. shasta* infection. Differences in susceptibility among chinook stocks have been demonstrated by Zinn et al. (1977) and Ching and Parker (1989), and differences in freshwater age composition among Fraser River chinook stocks are well recognized. In addition, the older fish may have a better developed immune system, thus enhancing their resistance to *C. shasta* infection.

Annual water temperature regimes apparently influence the timing of the seasonal onset of *C. shasta* infections within the Fraser River population of seaward migrating juvenile chinook salmon. This influence is presumably reflected in differences in the annual prevalence of *C. shasta*-infected fish leaving the river. The results of our sampling over the three years indicate that the earlier the water temperature in the lower Fraser River exceeded 10°C during the downstream migration period, the larger was the proportion of the population that became infected with *C. shasta*.

In early epizootiological investigations on ceratomyxosis of salmonids in California (Schafer 1968), temperatures above 10°C were considered essential for activation of the infective stage of *C. shasta* in spring. Although our observations on the onest of infection in relation to temperature are in accord with those of Schafer (1968), Ratliff (1983) reported that the Deschutes River system, a central Oregon tributary of the Columbia River, became infective as early as late March and at temperatures as low as 6.9°C. Similarly, Hendrickson et al. (1989) documented infectivity in the Klamath River, California, at 7°C in December, but the earliest infectivity in spring (April) coincided with a river water temperature of 15°C. Ching and Munday (1984a) also found that the lower Fraser River was infectious in November at temperatures between 4 and 6°C. It is possible that these low-temperature winter infections resulted from residual infective stages that had survived in the river from the previous spring and summer. Although temperature is important in influencing the rate of progress of ceratomyxosis and the onset of mortalities (Udey et al. 1975), it is also clear that temperatures in excess of 10°C are not always essential for initiating infections among salmonid populations in enzootic waters.

Infection prevalences (1.8–4.7% annually) recorded in our study of Fraser River juvenile chinook salmon are lower than the prevalences (8.9–14.0%) reported for chinook smolts captured in 1983 and 1984 in the lower Columbia River (Fryer 1984, 1985), suggesting that ceratomyxosis is less of a problem in the Fraser River than in the Columbia River. The reasons for the dissimilarity between the rivers are unknown, but environmental differences (e.g. dams on the Columbia River that reduce flow rates), along with stock susceptibility differences, may account for the disparity in the observed prevalences. Furthermore, we note that mortalities due to ceratomyxosis have occurred among juvenile salmonids in some hatcheries on the Columbia system (Conrad and Decew 1966; Wood 1979). In contrast, none of the juvenile chinook reared at hatcheries in the Fraser River system have been found to be infected with *C. shasta* at the time of release (G. Hoskins, Pacific Biological Station, pers. comm.).

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Whole-River Fertilization Stimulates Fish Production in an Arctic Tundra River

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Deegan, L. A., and B. J. Peterson. 1992. Whole-river fertilization stimulates fish production in an Arctic tundra river. Can. J. Fish. Aquat. Sci. 49: 1890–1901.

Increased growth rates for adult and young-of-the-year Arctic grayling (*Thymallus arcticus*) were measured after the addition of fertilizer to an oligotrophic Alaskan tundra river. The strongest response to the fertilization was seen in young-of-the-year grayling; the adult response was more variable. Whole-river phosphorus fertilization of the Kuparuk River, Alaska, during 1985–90 resulted in a 1.4- to 1.9-fold increase in the size of age 0+ fish and a 1.5- to 2.4-fold increase in the weight gain of adult grayling in some years. Neutral lipid storage in adult grayling was increased 1.3- to 3.4-fold in the fertilized zone compared with that in fish from the control zone. There was no detectable difference between the zones in gonad mass, percent lipid in eggs, or egg size. These results suggest that autochthonous production is an important energy source for fish in tundra streams and that nutrient limitation of stream ecosystems affects the highest trophic levels. These findings have importance for understanding the function of river ecosystems, for assessing human impacts on rivers, and for managing fisheries.

On a mesuré l'augmentation du taux de croissance des ombres arctiques (*Thymallus arcticus*) adultes et jeunes de l'année après ajout d'engrais dans une rivière oligotrophe de la toundra, en Alaska. C'est chez les jeunes de l'année qu'on a observé la réaction la plus forte; la réaction des adultes était plus variable. L'enrichissement en phosphore, à l'échelle de tout le cours d'eau, de la rivière Kuparuk (Alaska), entre 1985 et 1990, a provoqué une augmentation de l'ordre de 1,4 à 1,9 de la taille des poissons d'âge 0+, et une augmentation de 1,5 à 2,4 du gain de poids chez les adultes certaines années. Le stockage des lipides neutres chez les ombres arctiques adultes a augmenté d'un ordre de grandeur de 1,3 à 3,4 dans la zone enrichie par rapport aux poissons de la zone témoin. On n'a pas détecté de différences entre les zones en ce qui concerne la masse gonadique, le pourcentage des lipides dans les oeufs ou la taille des oeufs. Ces résultats semblent indiquer que la production autochtone est une source importante d'énergie pour les poissons dans les cours d'eau de la toundra, et que la limitation par les nutriants dans les écosystèmes lotiques a un effet sur les niveaux trophiques supérieurs. Ces découvertes ont une importance pour la compréhension de la fonction des écosystèmes lotiques, pour l'évaluation des effets anthropiques sur les cours d'eau et pour la gestion des pêches.

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Food availability and basic productivity of rivers and streams, which are often set by nutrient availability, are believed to be a major controlling factor in fish production in riverine ecosystems (McFadden and Cooper 1962). Despite this basic, and almost intuitive, premise the relationship between nutrient loading and populations at higher trophic levels in riverine ecosystems is poorly known (Peterson et al. 1981) and has rarely been studied in an experimental field setting (Warren et al. 1964; Elwood et al. 1981; Johnston et al. 1990). In lakes, increased nutrient loading, especially phosphorus input, usually results in increases in algal production and often, but not always, in secondary production of zooplankton and fish (LeBrasseur et al. 1978; Stockner 1987). The effects of experimental nutrient additions on river ecosystems have not been documented as extensively. Limitation of stream productivity by nutrients may be common where streams are not shaded (Stockner and Shortreed 1978; Peterson et al. 1981). In whole-river experiments, nutrient additions have been shown to stimulate epilithic algae (Elwood et al. 1981; Perrin et al. 1987; Johnston et al. 1990) and bacterial production (Peterson et al. 1985; Hullar and Vestal 1989). The response of insect populations is less well known, but accelerated development and increased body size for the dipterans *Orthocladius* and *Prosimulium* in the Kuparuk River (Hershey et al. 1988) and increases in density for the caddisfly *Brachycentrus* (Hershey and Hiltner 1988) have been documented.

The extent to which nutrient limitation in streams has effects on populations at higher trophic levels, such as fish, is unknown. The underlying mechanism of fertilization would be to increase algal productivity and production of aquatic insects which are a major food supply for fish. Several surveys have found that fish density and growth correlate with nutrient status and food supply in a stream (Murphy et al. 1981; Wilzbach 1985; Bowlby and Roff 1986). McFadden and Cooper (1962) found that streams carrying higher concentrations of dissolved solids supported a greater standing crop of brown trout (*Salmo trutta*) than those with lower ''fertility.'' Salmonid biomass at five stream sites in Newfoundland was apparently related to the type of habitat, stability of discharge, and level of nutrients (Gibson and Haedrich 1988). In addition, several experimental field studies support these observations. Warren et al. (1964) observed that a fourfold increase in invertebrate production, induced by adding sucrose to a stream, resulted in a sevenfold increase in the weight gain of young-of-the-year (YOY) trout in a hatchery stream. In this experiment, sucrose stimulated fungal growth which produced an increase in chironomid production. Mason (1976) increased the growth of YOY salmonids by supplemental feeding in a natural stream. Huntsman (1948)

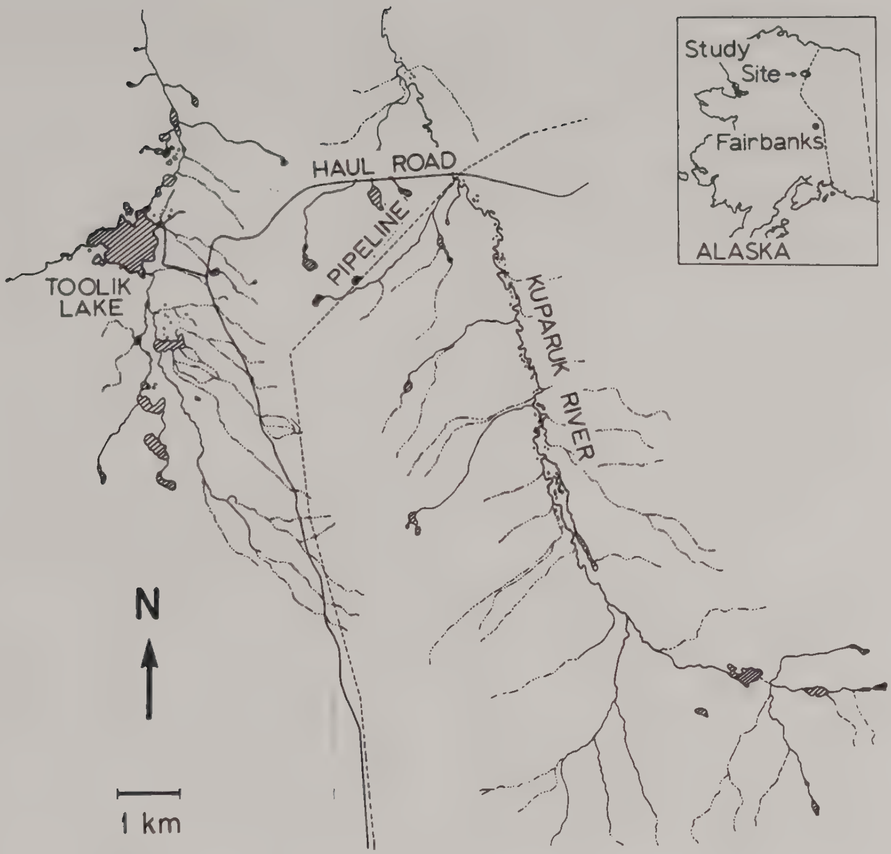

FIG. 1. Kuparuk River watershed. Hatched areas indicate headwater lakes; dotted lines indicate intermittent streams or small water tracks.

suggested that inorganic fertilization could increase fish production by stimulating aquatic insect growth. While there has been little work on the responses of fish to whole-stream nutrient fertilization, Johnston et al. (1990) have demonstrated that the addition of nitrogen and phosphate resulted in increased growth of young salmonids in the Keough River in British Columbia, Canada. Reed (1964) suggested that low levels of aquatic insect production limit the growth of Arctic grayling (*Thymallus arcticus*). Thus, nutrient additions to oligotrophic streams may be effective in stimulating fish production.

The Kuparuk River provided a favorable opportunity to test the hypothesis that increased nutrient availability would increase fish production in an oligotrophic system. Grayling is the only common species of fish in the upper reach of the Kuparuk River, and this fact greatly simplified the analysis of the fish response. Grayling use this section of the Kuparuk as a spawning and summer feeding area, allowing us to test the nutrient enrichment hypothesis on both adult and YOY (age 0+) fish. In streams, aquatic and terrestrial insects comprise most of the grayling diet (Reed 1964). Grayling apparently feed on whatever is available to them in the drift, although they occasionally forage on the stream bottom. In the Kuparuk River, their diet is over 80% aquatic insects, predominantly mayflies (*Baetis* spp.) and caddisflies (*Brachycentrus* spp.) (Hershey et al. 1993; Peterson et al. 1992a, 1992b). Because the Kuparuk River freezes solid during the winter with little water flow and few unfrozen pools, grayling migrate to upstream lakes to overwinter. Adult grayling leave these lakes soon after iceout in May or early June, move into the river, and spawn (Reed 1964; Beauchamp 1990). After spawning, they redistribute themselves and occupy summer feeding areas in late June or early July. During mid-August and early September, they migrate back to the lakes. YOY grayling emerge from gravel beds in late June or early July and apparently remain in the vicinity of where they hatch during their first 6–8 wk. The summer population of grayling in the experimental reach consists primarily of adults (6–10 yr old, 30–45 cm total length (TL), 300–650 g total weight (TW)) and age 0+ fish. The abundance of adults is roughly 50–100 fish per kilometre of river (three to five adults per pool). Individually tagged adult fish were often recaptured in August in the same pool they were initially captured from in July. YOY abundance varies widely from year to year from 50 to over 1000 per pool. Most juvenile grayling (1+ to 5+) spend the summer in lakes, tributary streams, or the shallow headwater section upstream of the experimental reach. They are normally not present in significant numbers in the experimental reach.

The purpose of this study was to demonstrate the effects of nutrient additions through bottom-up propagation through algae and insects to fish. If fish production is affected by nutrient addition, this finding has relevance in both understanding the controls on river trophodynamics and managing river fisheries.

TABLE 1. Mean daily river discharge and temperature of the Kuparuk River during the period of fertilizer addition (1 July to 20 August) each year. Discharge was measured with a continuously recording stage height monitor and converted to discharge using a stage height – discharge calibration curve. Temperature was monitored with a continuously recording thermistor, maximum–minimum mercury thermometers, and daily measurements. All temperature and discharge measurements were made at the road crossing which is in the middle of the experimental section. Temperature is not available for 1987 because floods destroyed the instrumentation.

Year	Mean discharge (m^3/s)	Mean temperature (°C)
1985	1.65	9.1
1986	2.74	9.4
1987	3.57	—
1988	1.22	10.1
1989	2.79	9.5
1990	0.32	12.1
Mean	2.07	10.0

Site Description

The Kuparuk River arises in the foothills of the Alaskan Brooks Range and flows north across the tundra of the north slope of Alaska to enter the Arctic Ocean west of Prudhoe Bay (Fig. 1). The experimental reach is near the headwaters of the Kuparuk on either side of the Dalton Highway (68°38′N, 149°24′W). In this stretch the river is a fourth-order meandering clearwater tundra stream draining rolling tundra with a substratum of rocky cobble and boulders. The upper river from the headwaters through our study site has a main channel length of 25 km, drains 143 km^2 of alpine and moist tundra communities, and has an average channel slope of 3% and sinuosity of 1.5 (Kriet et al. 1992). The vegetation of the upper drainage consists of alpine communities at higher elevations and moist tundra communities at lower elevations closer to the river. Along the stream banks, there are patches of dwarf willows and birches, some reaching 1 m in height. This experimental section of the river alternates between pools and riffles. The river basin is approximately 20 m wide, and the wetted surface in riffle sections is 50% and in pools is 75–80% at mean summer discharge. Riffle sections are about 30 m long, and at mean summer flows the water is 5–30 cm deep. Pools range between 1 and 2 m in depth in midsummer and are mostly 10–50 m long and 10–20 m wide. The control and fertilized reaches are similar in their physical attributes. Insect abundances were higher in the fertilized zone as a result of phosphorus addition (Hershey and Hiltner 1988; Hershey et al. 1988; Peterson et al. 1992b). The Kuparuk is frozen solid until May or June and has a high discharge in June due to snowmelt. River flow is lower in midsummer, although it responds quickly to intense rain or snow events. In late August, light and temperature decline sharply and the river freezes again in September or October.

Temperature and discharge characteristics were different every year of this study (Table 1). During the summers of 1985–90, water temperature ranged from 0.5 to 16°C and averaged 10°C. River discharge was measured continuously at the road crossing and during the period of river fertilization ranged between 0.2 and 30 m^3/s and averaged 2 m^3/s over 1985–90. Base summer flows are maintained by spring and lake discharge, while short flash floods are a response to local rain and snow events (Kriet et al. 1992). Discharge in 1990 was exceptionally low (average = 0.3 m^3/s) during the experiment and river temperatures were high (average = 12.1°C). During 1990, many riffles were almost dry and pools were isolated. A heavy rain event in early September filled the river and allowed fish to migrate to the headwater lake. Discharge in 1987 was high all summer with several flood events (>5 m^3/s). We were unable to maintain weirs in the river in 1987 because of the frequent flood events.

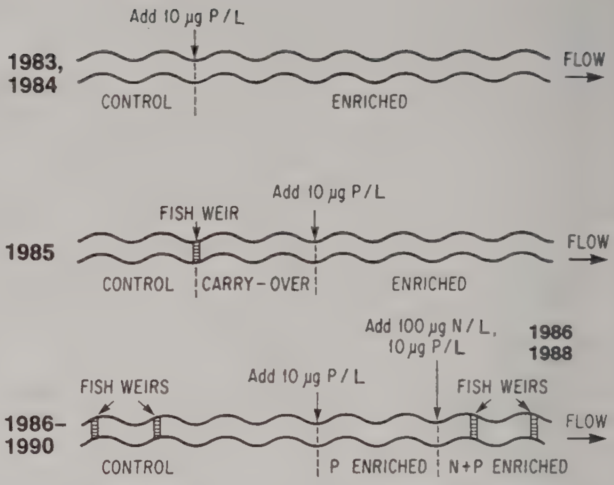

FIG. 2. Experimental design of whole-river fertilization experiment. Phosphorus fertilization began in 1983; the point of addition was moved downstream in 1985. The zone labeled "carry-over" was exposed to phosphorus addition during 1983 and 1984 and may have had some residual effects. This area was not used in the fish experiment.

Methods

Experimental Design

Nutrient addition

For seven consecutive years (1983–90), we added 10 µg P/L as phosphoric acid during 1 July to 15 August to a reach of the river accessible from the trans-Alaskan highway (Fig. 2). We present information on the response of fish for 1985–90; we have no fish information for 1983–84. Sampling stations were designated as plus (downstream fertilized reach) or minus (upstream control) kilometres in reference to the 1983 addition site. In 1985 the point of phosphorus addition was moved about 0.5 km downstream. This created a "carry over" area in the river during 1986 which we did not use because it might have shown some effects from phosphorus added in 1983–85. We have maintained the station location designations relative to the original site to be consistent with other publications. During 1986 and 1988, 100 µg N/L as ammonium sulfate was added in addition to a second addition of phosphorus at 1.7 km. The period 1 July to 15 August was chosen as the fertilization period because it spans the time of warmest waters and highest activity at all trophic levels during the brief arctic summer.

Fish experiment design (1985–90)

The response of the fish to nutrient addition was monitored by comparing individually tagged fish in the fertilized reach with individually tagged fish from the control reach after 4–6 wk of fertilization. In 1985, the fertilized and control reaches were separated in mid-July by a nylon fish weir with a 5-cm mesh that prevented adult fish from moving between the zones. Recaptures of tagged fish in the river and observations on fish held in tanks indicate that this mesh size effectively

TABLE 2. Mean initial and final size and condition (± SE) of all experimental fish for 1986, 1988, 1989, and 1990 for the Kuparuk River. Two-way ANOVA indicated no difference in initial length, weight, or condition factor for fish residing in the fertilized and control sections for any year ($p < 0.01$). The mean change presented in Fig. 3 was determined by taking the difference between initial and final values of individual fish and averaging the differences.

			Initial		Final	
Year	Zone	Number of fish	Mean	SE	Mean	SE
			Total length (cm)			
1986	Control	23	36.0	0.4	36.3	0.4
	Fertilized	17	37.3	0.7	37.1	0.7
1988	Control	17	36.5	0.5	36.7	0.5
	Fertilized	13	36.6	0.9	36.9	0.8
1989	Control	8	36.3	0.6	36.7	0.6
	Fertilized	45	36.0	0.2	36.9	0.2
1990	Control	8	35.0	1.2	35.4	1.2
	Fertilized	15	36.6	0.5	36.9	0.5
			Total weight (g)			
1986	Control	23	364	12.8	396	12.2
	Fertilized	17	417	21.3	471	21.3
1988	Control	17	401	15.3	407	14.7
	Fertilized	13	421	24.8	427	22.9
1989	Control	8	377	17.0	423	20.7
	Fertilized	45	364	6.6	434	8.1
1990	Control	8	385	30.6	383	31.3
	Fertilized	15	445	17.6	453	16.3
			Condition factor			
1986	Control	23	0.77	0.01	0.82	0.01
	Fertilized	17	0.79	0.01	0.85	0.01
1988	Control	17	0.82	0.01	0.81	0.01
	Fertilized	13	0.84	0.01	0.84	0.01
1989	Control	8	0.78	0.01	0.85	0.02
	Fertilized	45	0.77	0.01	0.86	0.01
1990	Control	8	0.89	0.02	0.85	0.02
	Fertilized	15	0.89	0.01	0.90	0.01

TABLE 3. Two-way ANOVA analyses of the growth response of grayling to fertilization (Fig. 3). Zone = control and fertilized reaches; Year = 1986, 1988, 1989, and 1990.

Source	df	Sum of squares	Mean square	*F*-value	*p*-value
Change in total length (cm)					
Year	3	2.298	0.766	4.702	0.0037
Zone	1	0.913	0.913	5.601	0.0193
Year·Zone	3	1.405	0.468	2.875	0.0385
Residual	138	22.486	0.163		
Change in total weight (g)					
Year	3	60774.706	20258.235	33.250	0.0001
Zone	1	5349.097	5349.097	8.780	0.0036
Year·Zone	3	2702.055	900.685	1.478	0.2232
Residual	138	84079.153	609.269		
Change in condition factor					
Year	3	0.170	0.057	26.488	0.0001
Zone	1	0.007	0.007	3.067	0.0821
Year·Zone	3	0.004	0.001	0.505	0.6798
Residual	138	0.296	0.002		

prevents fish >25 cm from passing through the weir. Adults were collected in August by seining and angling from each reach for an end-of-summer comparison. Only end-of-summer comparisons were possible on adult fish in 1985. In subsequent years, all data are on individually tagged individuals measured at the beginning and end of the experiment. Fish were allowed to redistribute themselves into summer feeding areas in June, and then segments of the control (−0.1 to −1.5 km) and fer-

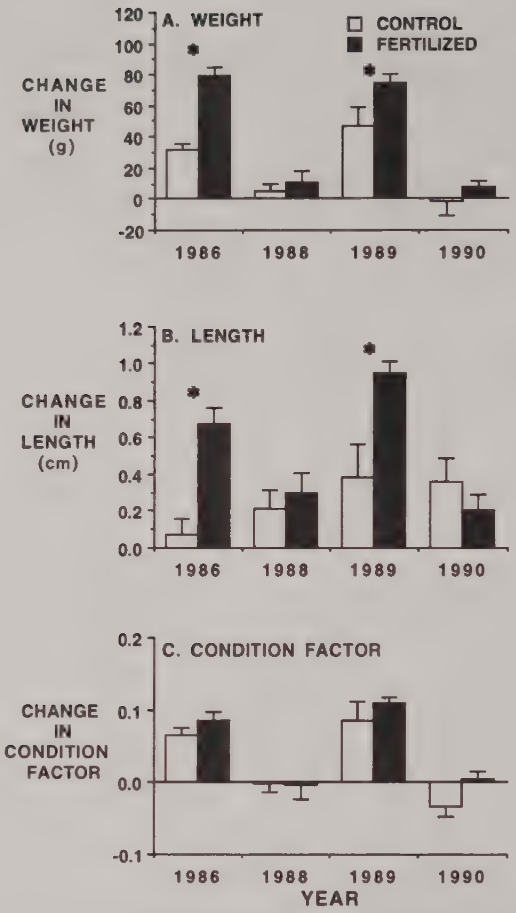

FIG. 3. Growth of adult grayling in the control and fertilized reaches for 1986–90. (A) Mean (±SE) total weight change; (B) mean (±SE) total length change; (C) mean (±SE) condition factor change. Two-factor ANOVA analyses are presented in Table 4; significant ($p < 0.05$) contrasts indicating differences between zones within years are indicated with asterisks.

tilized reaches (+2 to +3 km) were isolated by black plastic fish weirs at the beginning of July. Adult fish in both sections were tagged, weighed, measured, and released during the first week of July. In mid-August, tagged fish were recaptured and measured. Some fish were shipped frozen to the laboratory for additional analysis of sex, lipid content, and fecundity. Sex could only be reliably determined on dissected fish.

Densities of adult and YOY grayling varied between years. Estimates of adult fish densities in experimental reaches based on recaptures during the experiment and above- and below-water visual surveys were approximately 100 fish/km in 1986, 50 fish/km in 1988, and an unequal distribution of 15 fish/km in the control reach and 120 fish/km in the fertilized reach in 1989. We have no information on grayling from 1987 because floods removed the weirs separating the two reaches. Abundance of YOY grayling was patchy and variable between years. YOY grayling were present in schools ranging from 10–20 to several hundred.

Measurement of growth (adults)

In the field, anesthetized fish were measured (TL, ±0.5 cm) and weighed (TW, ±1 g) at the beginning and end of the experiment. In 1985, only TL was measured. In 1986, weights were taken immediately after capture. In 1988 and 1989, fish were weighed after being held in cages for 24 h to evacuate their guts. Fultons condition factor (Cf) was used as an estimate of condition (Bolger and Connolly 1989).

Changes in TL, TW and Cf were calculated as the difference between initial (July) and final (mid-August) values for individually tagged fish in each reach. Only fish tagged in the beginning and recaptured at the end of the experiment were used in this analysis. The length of time fish were at large in the river varied between 28 and 35 d; change was not corrected for the number of days at large. Initial and final capture efforts were alternated between zones so there was no consistent bias in time at large for fish in the two zones.

Measurement of growth (YOY)

Age 0+ grayling were sampled shortly after they emerged from the gravel in July and then again in mid-August. Age 0+ fish were collected with dip nets and seines and taken to the field laboratory for determination of TL (±0.1 mm measured with calipers) and wet weight (±0.001 g). In 1985, only length was measured; weights were estimated from site-specific length/weight regressions developed for larvae in 1986. As the YOY became larger or at high river discharge, YOY became more difficult to capture. High river discharge also made it difficult to capture age 0+ fish. Usually 20–50 individuals were taken from a single station. Fish were not replaced, but this did not seem to affect abundance. YOY fish were captured from several sampling locations in the control and fertilized reaches, and data were combined within a reach to give a mean as recommended by Hurlbert (1984) and Stewart-Oaten et al. (1986).

Body composition of adults

Fish were defrosted, sexed, and then dissected to remove liver, gonads, and the intestine with fat attached. From females in 1988 and 1989, one gonad was used for lipid determination and the other was stored in Gilson's solution for fecundity analysis. Organs were placed in separate preweighed containers, weighed (±0.001 g), and dried to constant weight at 60°C (usually 48 h). The containers were removed from the oven and placed immediately in a desiccator to cool and then dry weights were determined.

Energy storage was evaluated by measuring neutral lipid storage in the intestinal area. As is typical of salmonids, the major storage of energy in grayling is in lipid deposited in the intestinal area. Initial extractions of lipids from the intestinal area, muscle, and liver indicated no appreciable storage of lipid in the liver (<1% of total storage) or the muscle (<5% of total storage), so these analyses were not continued. Large quantities of fatty material were present in the intestinal area and represented up to 50% of the weight of tissue in this area and 94% of total neutral lipid storage. Lipid stored in eggs was not considered part of available energy reserves.

Weight of neutral lipid surrounding the intestine was determined using hexane extraction (Gardiner and Geddes 1980; Ohman 1988). After weighing, intestines were covered with 10 volumes of hexane, crushed to a fine powder using a glass ceramic mortar and pestle, covered tightly, and stored in a hood for 24 h. The hexane was decanted and replaced; this was repeated three times or until the hexane removed was clear. The tissues were dried for 48 h, cooled in a desiccator, and weighed again. Neutral lipid weight is the difference between dry weight and weight after hexane extraction.

The liver, which functions as a storehouse of glycogen and produces vitellogellin which is important in egg development, provides a shorter term index of nutritional state. The hepatosomatic index (HSI) is calculated as HSI = (liver weight × 100)/TW, where TW is total weight.

Reproductive response was measured several ways. Total wet mass of gonad tissue was determined and used as an estimate of immediate response to fertilization. Lipid content of eggs was determined using the lipid extraction method outlined above. After dissecting the fish, one female gonad was weighed and placed in Gilson's fluid (Bagenal and Braum 1968). Samples were shaken periodically to remove ovarian tissue; samples were then drained, rinsed, and weighed. Four subsamples were taken using a plankton splitter and each subsample was weighed. Eggs were counted under a dissecting scope and the mean number of eggs per gram was calculated. To determine mean egg diameter, a random sample of 150 eggs was measured at 6.5 magnification (Mann and Mills 1985). Mean egg size was calculated by treating the eggs as spheres.

Our approach to data analysis was to use a modification of the "before–after control–impact" design (Stewart-Oaten et al. 1986) by considering the July and August size data as "before" and "after," respectively. Unfortunately, we have no quantitative information on the size distribution of grayling in the control and fertilized zones prior to fertilization. Ancedotal information from fishermen prior to fertilization indicates no obvious differences in size or distribution of grayling between the control and fertilized reaches. We have 2 yr of "prior-to-fertilization" information on a second similar river we started fertilizing in 1991 and we have found no differences in grayling size between control and fertilized zones.

Data was analyzed using a two-factor analysis of variance (ANOVA) design. The first factor was treatment zone (control or fertilized); the second was year of experiment. The number of years varied with the measurement. Growth of individual fish during the summer was measured in 1986, 1988, 1989, and 1990. The numbers of fish recaptured each year in the control and fertilized reaches, respectively, were 23 and 17 (1986), 17 and 33 (1988), 8 and 45 (1989), and 15 and 8 (1990). At the beginning of the experiment, two-factor ANOVAs and contrasts indicated that average length, weight, and condition were not significantly different for fish residing in the fertilized and control reaches in any year (Table 2, $p < 0.01$). End of the summer determination of body composition was done in 1985, 1986, and 1988, except fecundity and egg size which were measured only in 1988 and 1989. Because of small sample sizes within each year, data on fecundity and egg size were combined into a one-factor ANOVA with zone as the main effect. Contrasts were used to determine differences within a year between zones.

Results

Changes in Weight, Length, and Condition of Adults

The mean weight change in the fertilized reach was significantly higher than the weight change in the control reach (4-yr averages: 55 and 24 g in fertilized and control, respectively) (Fig. 3; Table 3). There was also a significant difference in overall response with year (Table 3). The most striking difference in growth between the two reaches was in 1986, when adult grayling in the fertilized reach gained almost twice as much weight during July and August compared with fish in the control zone (79 versus 32 g, in the fertilized and control reaches, respectively; Fig. 3). In fact, no fish in the control reach gained as much weight as the average fish in the fertilized reach in 1986. In 1988, growth was quite low and although the fish in the fertilized reach had a slightly higher average weight gain (8 g) than fish in the control zone (5 g) the difference was not significant. In 1989, overall weight gain returned to levels similar to 1986, and fish in the fertilized reach gained about 1.5 times as much weight as fish in the control zone (70 versus 46 g, respectively). The higher growth in the fertilized zone was sustained despite an almost sixfold increase in density of fish in the fertilized zone (120 fish/km) compared with the control zone (15 fish/km) in 1989. In 1990, grayling in the control zone lost weight, while fish in the fertilized zone gained weight slightly.

Changes in length were usually higher in the fertilized reach compared with the control, although changes were not large (Fig. 3; Table 3). In 1986, fish in the fertilized reach increased 0.7 cm in TL compared with 0.1 cm for the control fish. In 1988, the change in length was similar for both reaches (0.3 versus 0.2 cm in the fertilized and control reaches, respectively). The change in length in 1989 was similar to 1986, with fertilized fish gaining three times as much in length (0.9 versus 0.3 cm, respectively). In 1990, fish from both zones increased in length approximately 0.2 cm.

The change in Cf was not large and was more influenced by year than zone (Fig. 3; Table 3). The change in Cf was highest in the fertilized zone compared with the control zone (0.09 versus 0.06, respectively) in 1986. In 1988, fish in both the control and fertilized zones lost condition slightly (<0.01 each) (Fig. 3). The change in condition of fish in the fertilized zone was slightly higher than the control zone in 1989 (0.11 versus 0.08, respectively) and the overall level was similar to 1986. The change in Cf in 1990 was low and similar to 1988, with a decrease in the control zone (−0.02) and a slight increase in the fertilized zone (0.01).

Available Energy Storage and Reproductive Response

Neutral lipid storage by fish in the fertilized reach was almost double that of fish from the control reach for both males and females in all years (Fig. 4; Table 4). For females, the average weight of lipid stored in the intestinal area was approximately 2.1 and 0.9 g for fish from the fertilized and control reaches, respectively. Two-factor ANOVA indicated a significant effect of zone but no difference in neutral lipid storage between years for females (Table 4). For males the average lipid weight was slightly lower than for females, 1.9 and 0.9 g for fish from the fertilized and control reaches, respectively. Two-factor ANOVA indicated a strong effect of zone and a slight effect of year with highest neutral lipid storage in 1986 and 1989 for males (Fig. 4; Table 4).

The HSI at the end of the summer was not different between zones for either females or males (Fig. 5, Table 4). Females usually had HSI values near or above 1, while males tended to have values slightly below 1. Both female and male HSI values were different between years (Table 4). Interestingly, in 1988 when overall growth was low, the HSI did not show a concomitant decline in either males or females (HSI = 0.7 and 1.1, respectively). The lowest HSI values were in 1985 when there was no difference between males and females (HSI = 0.5).

Reproductive Response

There was no difference in gonad mass between zones for either females or males (Fig. 6; Table 4). The overall average weight of ovarian tissue was 6.7 and 6.8 g for fish from the fertilized and control reaches, respectively. The difference between the reaches was most striking in 1988 when overall ovarian weight was highest (14 and 8 g for fertilized and control

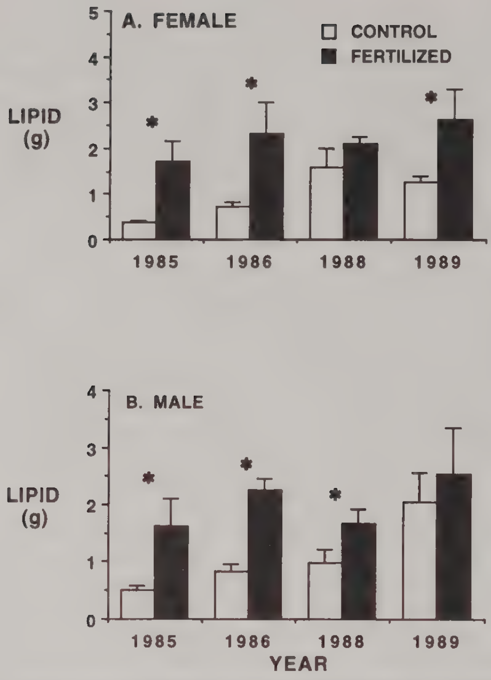

FIG. 4. Mean (±SE) neutral lipid storage at the end of the summer for adult grayling from the control and fertilized reaches for 1985–89. (A) females; (B) males. Two-factor ANOVA analyses are presented in Table 4; significant ($p < 0.05$) contrasts indicating differences between zones within years are indicated with asterisks.

reaches, respectively) and TW gain was low (Fig. 3). The high value in the fertilized zone for 1988 represents only two females and has a high variance. The pattern of slightly higher values in the fertilized reach compared with the control was found in other years; however, this difference was small and not significant in most years (Fig. 6). Males had an average gonad weight of 4 g regardless of fertilization or year (Fig. 6).

There was no apparent change in lipid content of ovarian tissue, mean number of eggs per gram, or egg size in response to river fertilization (Fig. 7). Lipid content of ovarian tissue did not vary among years or between treatments and averaged 11%. Egg number and size was measured on six females in 1988 (five control and one fertilized) and 10 females in 1989 (one control and nine fertilized). The size of eggs was not different between the two reaches when both years were combined; however, given the high variabilities, the sample size was probably too small to detect a difference if it existed.

Growth of YOY

The average effect of fertilization was a 1.3- to 1.9-fold increase in mean weights of YOY fish over the mean weight of fish in the control zone (Fig. 8). Grayling emerged from the gravel in early July and there was no difference in larval size between these reaches in the earliest collection. For example, weight of larvae on 4 and 5 July 1986 averaged 0.15 and 0.18 g from fertilized and control reaches, respectively. By August 1986, however, there was a highly significant difference between the mean weights of age 0+ grayling from the control and fertilized reaches. YOY fish from the fertilized reach were 40% heavier than YOY fish from the control reach (1.06 versus 1.41 g in control and fertilized reaches, respectively). In fact, by the end of the summer of 1986, it was rare to find age 0+ fish in the control reach that were as large as the average fish in the fertilized reach (Fig. 8). The difference between the control and fertilized reaches was even more apparent in subsequent years. By mid- to late July, there was little overlap in sizes of young fish between the zones in any year (Fig. 8). The difference in mean size of YOY was consistent throughout the two reaches of the river (Fig. 9). Samples taken from 10 stations in 1986 indicated a consistent difference in YOY weight between the control (1.1 g) and fertilized (1.4 g) reaches.

There were also differences in average size of YOY fish between years (Fig. 8) which seem related to river flow and temperature. Although differences in exact date of sample make direct comparison among all years difficult, YOY grayling seem to grow fastest in years of low flow and warm temperatures. Comparison of 1988, a moderately low-flow (1.22 m^3/s) and warmer temperature (10.1°C) year, with 1989, a moderately high-flow (2.79 m^3/s) and slightly cooler temperature (9.5°C) year, illustrates this point (Fig. 8). By 14 July 1988, YOY grayling averaged 0.71 g across both zones. By mid-July 1989, the average weight of YOY grayling was only 0.12 g. In 1990, a very low-flow and high-temperature year, fry sizes were the largest of any year in our study. Thus, like the adult fish response, the effect of fertilization seems to be superimposed upon a year-to-year variation in growth influenced by discharge and temperature.

Discussion

Both YOY and adult grayling have experienced increases in growth and condition following nutrient addition. These results show the effects of "bottom-up" propagation of nutrients through algal and invertebrate levels to fish. The strongest, most consistent response was the increase in size of YOY grayling. Adults increased neutral lipid storage in all years, growth in most years, and possibly reproductive potential through larger body size.

These responses were presumably due to the greater size and number of food organisms such as *Baetis*, *Brachycentrus*, and small chironomids in the fertilized reach (Hershey and Hiltner 1988; Hershey et al. 1988). Both *Baetis* and *Brachycentrus* are important food items for adult grayling (Hershey et al. 1993; Peterson et al. 1992b). *Baetis* production may be particularly important to grayling which feed heavily on emerging *Baetis* during late July and August as they prepare for their upstream migration to overwintering lakes. Shifts in stable carbon and nitrogen isotope distributions clearly demonstrate the coupling between the stimulation of benthic algal photosynthesis and the accelerated growth of all the insects and grayling (Peterson et al. 1992a, 1992b).

The immediate increases in YOY grayling size during fertilization suggested that growth was food limited. Several studies have indicated that growth rates of young salmonids are food limited. Johnston et al. (1990) increased the growth of steelhead (*Oncorhynchus mykiss*), coho (*O. kisutch*), and Dolly Varden (*Salvelinus malma*) fry with nutrient enrichment, presumably through increasing food availability. Murphy et al. (1981) found that growth rates of salmonid fry varied directly with insect drift in several Oregon streams. Mason (1976) found that both the size and density of juvenile coho salmon were increased severalfold by experimental feeding.

The response of adult fish indicates that energy storage and growth are also food limited. This confirms the results of many surveys that have found positive correlations between nutrient concentrations and fish production (McFadden and Cooper

TABLE 4. ANOVA analyses of the body composition response of grayling to river fertilization (Fig. 4–7). Zone = control and fertilized reaches; Year = among years (1985, 1986, 1988, and 1989).

Source	df	Sum of squares	Mean square	F-value	p-value
Male gonad (g)					
Zone	1	0.1	0.1	0.01	0.96
Year	3	38.0	12.6	4.6	0.001
Zone·Year	3	27.3	9.1	3.3	0.02
Residual	56	152.1	2.7		
Female gonad (g)					
Zone	1	18.0	18.0	2.3	0.12
Year	3	158.3	52.7	6.9	0.001
Zone·Year	3	49.3	16.4	2.1	0.10
Residual	51	387.0	7.6		
Male neutral lipid (g)					
Zone	1	9.9	9.9	12.6	0.001
Year	3	6.7	2.2	2.8	0.04
Zone·Year	3	1.9	0.6	0.8	0.47
Residual	56	43.9	0.7		
Female neutral lipid (g)					
Zone	1	10.7	10.7	6.3	0.01
Year	3	6.6	2.2	1.3	0.28
Zone·Year	3	1.5	0.5	0.3	0.81
Residual	51	86.2	1.6		
Female HSI					
Zone	1	0.1	0.1	2.4	0.12
Year	3	2.9	0.9	17.3	0.001
Zone·Year	3	0.1	0.1	1.0	0.39
Residual	53	2.9	0.1		
Male HSI					
Zone	1	0.0	0.04	1.2	0.26
Year	3	1.1	0.38	11.8	0.001
Zone·Year	3	0.2	0.08	2.4	0.07
Residual	56	1.8	0.03		
Female % gonad as neutral lipids					
Zone	1	52.1	52.1	1.5	0.21
Year	3	126.5	42.1	1.2	0.29
Zone·Year	3	25.3	8.4	0.3	0.85
Residual	53	1778.7	33.5		
Size mean egg (mm)					
Zone	1	0.446	0.446	0.21	0.65
Residual	14	29.468	2.105		
Fecundity (no. of eggs/g)					
Zone	1	2.6×10^6	2.6×10^6	0.52	0.47
Residual	14	70×10^6	5×10^6		

1962; Egglishaw 1968; Gibson and Haedrich 1988). There was no immediate difference in reproductive potential between the control and fertilized zones, although there may be a long-term response because of increased size. Neither females nor males had a higher gonad mass in the fertilized zone; lipid content and size and number of eggs did not differ between the zones. In other fish, there is no evidence that changes in either the size or chemical composition of eggs were due to either the quantity or quality of food available (Wootton 1985). It is possible that fish tend to maintain the quality of the eggs and alter fecundity or the number of spawnings.

If production of grayling were limited solely by food availability in the mainstem stream during the summer, the long-term response to fertilization should be an increase in the total population size (Fig. 10). This is a result of several factors operating on both adult and young grayling. During the summer of fertilization, the immediate adult response is an increase in growth rate and neutral lipid storage. The increase in neutral lipid storage should lead to higher overwintering survival because the grayling have more energy stored for use during the seven to eight winter months when aquatic production of food resources is very low or nonexistent. During this time, grayling are inactive and rest near the bottom existing primarily on stored energy reserves. The increased summer growth rate leads to a larger overall size at age for both male and female grayling. A larger body size is advantageous in feeding hierarchies and defending territory by males in the following spring (Beauchamp 1990; Hughes and Dill 1990). The increased body size and neutral lipid storage would both be expected to increase the survivorship of adults leading directly to a larger population size. In addition, there are some indirect longer term feedbacks. In females, fecundity increases as a power function with body

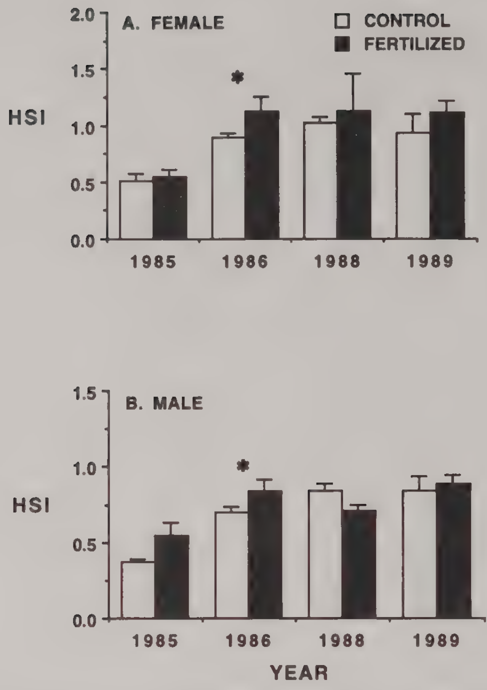

FIG. 5. Mean (± SE) hepatosomatic index (HSI) at the end of the summer for adult grayling from the control and fertilized reaches for 1985–89. (A) females; (B) males. Two-factor ANOVA analyses are presented in Table 4; significant ($p < 0.05$) contrasts indicating differences between zones within years are indicated with asterisks.

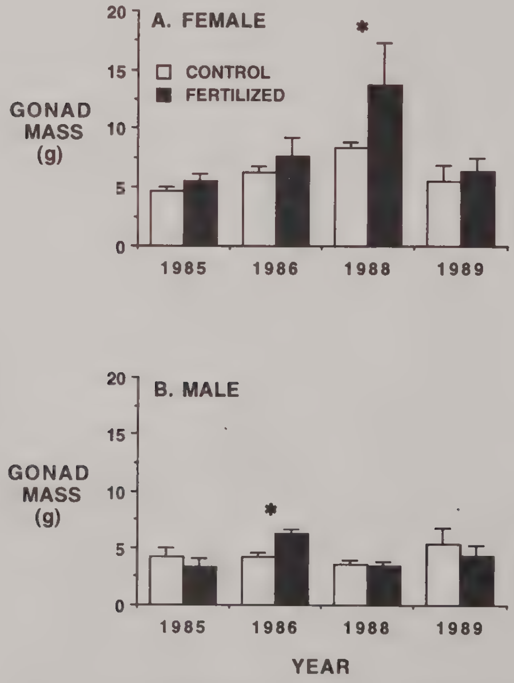

FIG. 6. Mean (± SE) gonad mass at the end of the summer for adult grayling from the control and fertilized reaches for 1985–89. (A) females; (B) males. Two-factor ANOVA analyses are presented in Table 4; significant ($p < 0.05$) contrasts indicating differences between zones within years are indicated with asterisks.

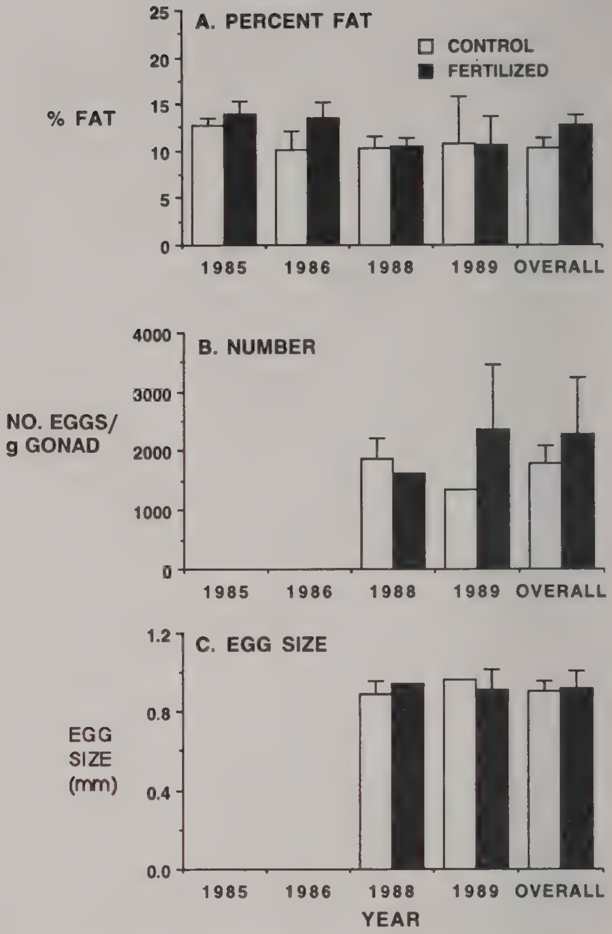

FIG. 7. Egg quality and fecundity at the end of the summer for adult grayling from the control and fertilized reaches for 1985–89. (A) Mean (± SE) percent lipid in eggs; (B) mean (± SE) number of eggs per gram of gonad; (C) mean (± SE) size of eggs. One-factor ANOVA analyses on data combined over years indicated no significant difference between zones (Table 4).

size (Wootton 1985; L. A. Deegan, unpubl. data) leading to higher egg production in subsequent years because of the larger size attained as a result of increased growth.

The response in YOY fish is primarily increased growth resulting in a larger size throughout the summer. We expect these size differences in YOY fish to be important for future recruitment because increased size usually means increased survival. For example, experiments in which hatchery pink (*Oncorhynchus gorbuscha*) or chum (*O. keta*) fry were fed to achieve a larger size at release have increased survivorship to adult, in some cases as much as doubling the number of returning adults (Martin et al. 1981). One reason for this is that swimming speed, which affects the ability to secure food and escape predators, increases sharply with increased size of fish (Scott 1985; Simonson and Swenson 1990). Experiments with Pacific salmon have shown that predation intensity is highest on smaller fry (Parker 1971). Very small differences in size of larval coho salmon (32 versus 27 mm standard length) result in 70 versus 40% survivorship, respectively, because of differences in swimming performance. This should increase survivorshp of YOY because burst swimming speed increases with length (Beamish 1978; Scott 1985; L. A. Deegan, unpubl. data).

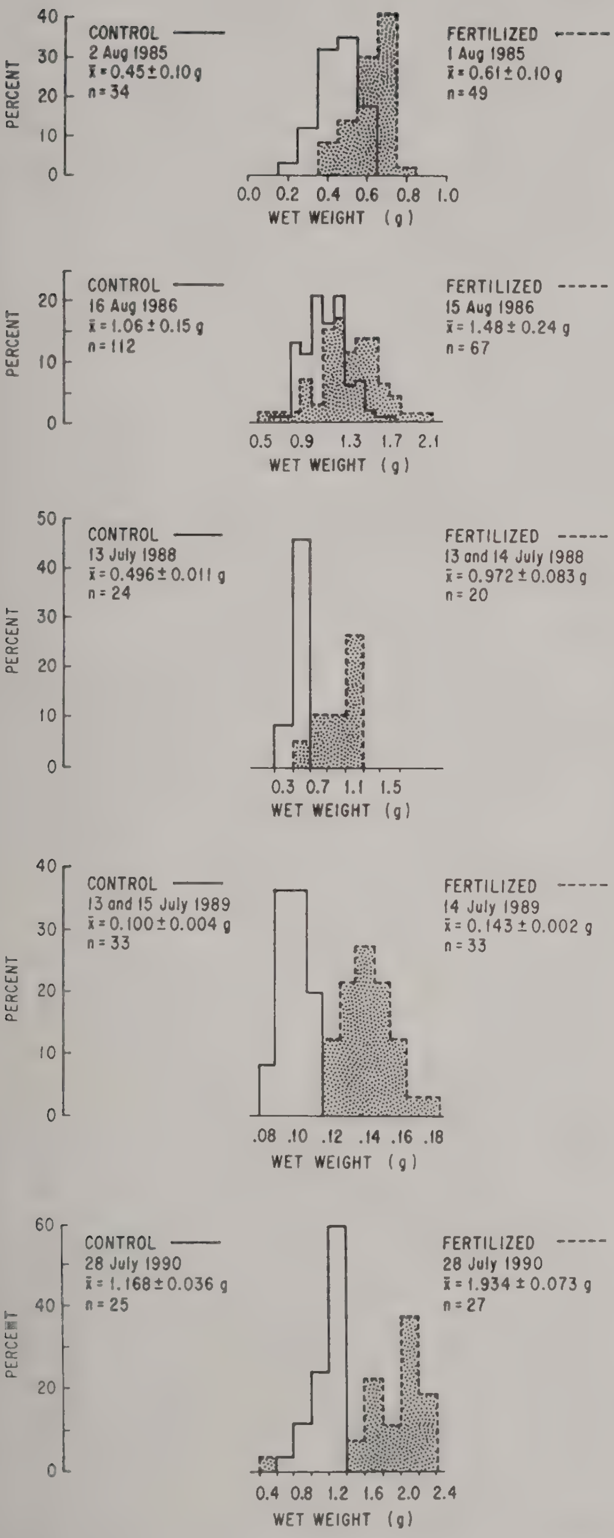

FIG. 8. Mean (±SE) weight of age 0+ grayling at the end of summer from the control (open bars) and fertilized reaches (stippled bars) for 1985–90.

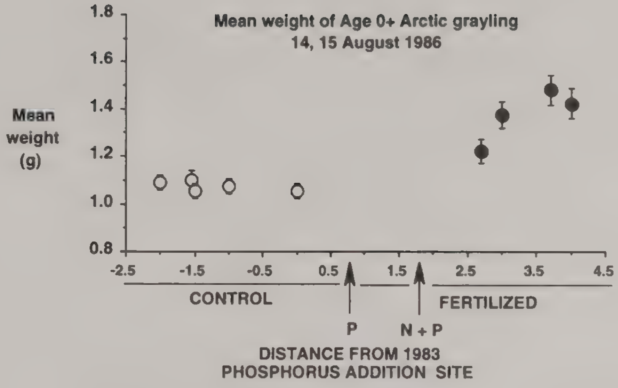

FIG. 9. Mean (±SE) weight of age 0+ grayling at the end of the summer (1986) along a transect from the control (open circles) to the fertilized reach (solid circles).

Increased burst swimming speed should increase the ability of grayling to feed in faster currents, to escape predation, and to resist being washed downstream in floods. All of these factors should increase the first year survival of the larger, more robust young grayling from the fertilized reach. The long-term consequences of better survival in the first year should be a long-term increase in the number of young grayling that reach adult size.

Increased population size should also have some negative feedbacks which would likely limit the ultimate size of the grayling population. Salmonid growth is highly influenced by food availability and fish density (Murphy et al. 1981; Randall 1982; Wilzbach 1985). Eventually, increased density of grayling should lead again to food-limited growth. At high adult fish densities, availability of adequate territorial sites for spawning and feeding may also limit production (Beauchamp 1990; Hughes and Dill 1990). If the number of spawners increases, poor reproductive success might result from either superimposed spawning or increased behavioral interference among males (Beauchamp 1990). The energy costs of holding a reproductive territory under high population densities may be significant (Wootton 1985). Aggressive behavior also establishes feeding territories and determines occupation of prime feeding locations (Reed 1964; Hughes and Dill 1990). Availability of overwintering habitat is another potential limit on the increase in grayling population size. Because these rivers freeze solid in the winter, grayling must move to lakes to overwinter. Increased oxygen demand due to higher biomass (more, larger sized fish) may cause winter fish kills due to lack of oxygen under the ice.

Total population size may also be limited by factors not affected by fertilization. For instance, age 1–5 grayling live in lakes or smaller streams. We do not know what factors control their mortality but predation, floods, or drought may ultimately limit the conversion of YOY to adult grayling.

It is important to note that our fertilization experiment affected only a small portion of the river which is used by this grayling population (5 km out of 100 km) and that age 1–5 fish do not usually use this section of the river, so it is not likely that we will actually see a long-term increase in the size of this population.

The growth rate of grayling varied widely from year to year. The difference in average TW gain for both the fertilized and control sections varied from essentially zero in 1990 (extremely low flow and high temperatures) to around 50 g in 1986 and

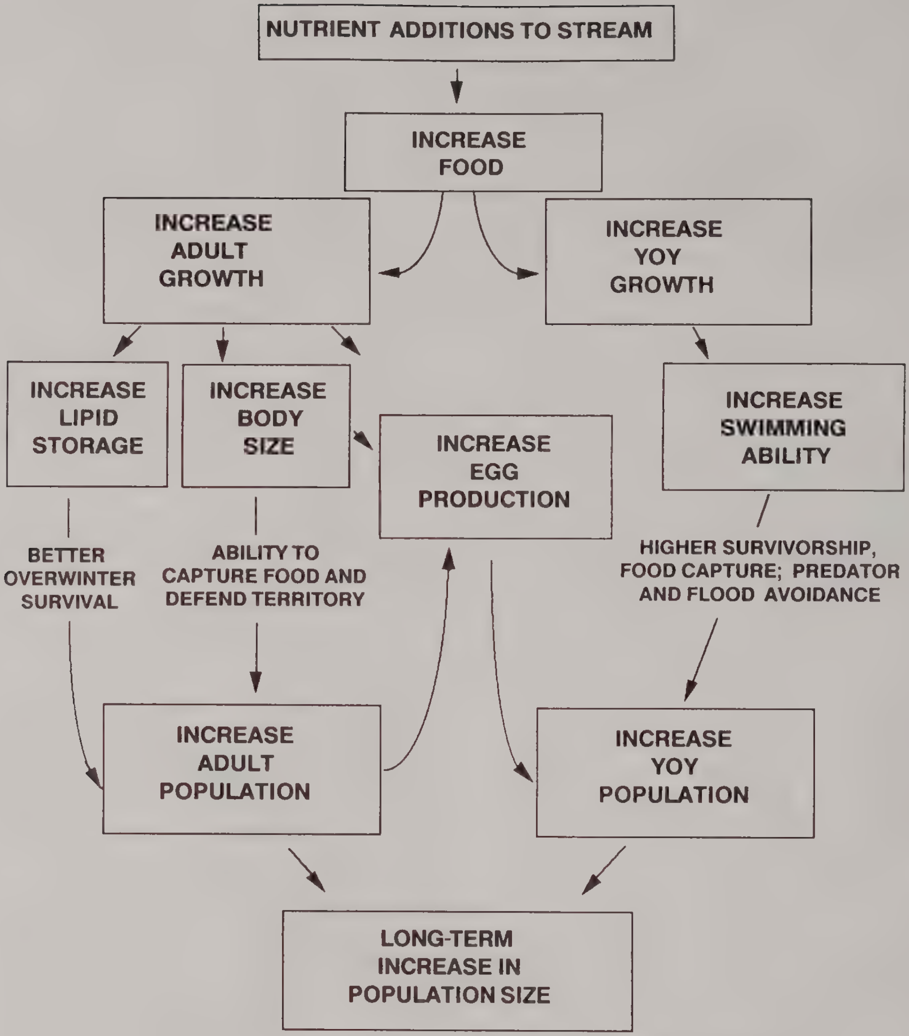
NUTRIENT ADDITIONS TO STREAM
INCREASE FOOD
INCREASE ADULT GROWTH
INCREASE YOY GROWTH
INCREASE LIPID STORAGE
INCREASE BODY SIZE
INCREASE EGG PRODUCTION
INCREASE SWIMMING ABILITY
BETTER OVERWINTER SURVIVAL
ABILITY TO CAPTURE FOOD AND DEFEND TERRITORY
HIGHER SURVIVORSHIP, FOOD CAPTURE; PREDATOR AND FLOOD AVOIDANCE
INCREASE ADULT POPULATION
INCREASE YOY POPULATION
LONG-TERM INCREASE IN POPULATION SIZE

FIG. 10. Overview of the response of grayling to nutrient additions to the stream.

1989. The effect of fertilization was superimposed upon a level of stream productivity that is influenced by flow and temperature regimes which we are only beginning to understand. Low flow and higher water temperatures affect the energy balance of grayling by decreasing insect drift density (LaPerriere 1983) and increasing metabolic respiration demands (Tytler and Galow 1985). The importance of fertilization in increasing grayling growth seemed to be most evident in years of moderate flow and medium temperatures such as 1986 and 1989. In years of low flow and higher temperatures (1988 and 1990) the difference in growth between the two zones is not as apparent. Unfortunately during the year (1987) of high river flow and low temperature, our weirs were destroyed by floods, so we do not have information on growth of grayling under these conditions.

The response of grayling to whole-river fertilization has several implications. First, addition of small amounts of nutrients to an extremely oligotrophic river has increased overall productivity to the highest trophic level. The grayling response is a clear example of ''bottom-up'' regulation of fish growth in a river ecosystem. The addition of nutrients propagated through the entire system from algae to insects to fish. This is important for predicting the consequences of human development or disturbance which mobilize nutrients in these watersheds.

Second, while we do not yet know all the long-term consequences of fertilization for grayling populations, our results indicate that addition of small amounts of nutrients may be a way to enhance fisheries production in nutrient-poor rivers if nutrient additions to a whole watershed can be managed. While it might take a decade or more of a scaled-up fertilization regime to see the full population level responses to fertilization, our results indicate that fertilization has affected many aspects of grayling life history, including age 0+ and adult growth, and energy stores available for migration and overwintering survival, and possibly reproductive output. Experiments in other arctic rivers and nutrient-poor temperate rivers would test the generality of these results and provide the information needed to evaluate the fertilization option for enhancing managing fisheries in oligotrophic running waters.

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Mitochondrial DNA Diversity among Lake Superior and Inland Lake Ciscoes (*Coregonus artedi* and *C. hoyi*)

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Snyder, T. P., R. D. Larsen, and S. H. Bowen. 1992. Mitochondrial DNA diversity among Lake Superior and inland lake ciscoes (*Coregonus artedi* and *C. hoyi*). Can. J. Fish. Aquat. Sci. 49: 1902–1907.

Mitocondrial DNA (mtDNA) of ciscoes from Lake Superior and seven inland lakes in Michigan and New York were analyzed for restriction fragment length polymorphism using 11 informative restriction endonucleases. Five of the 11 enzymes revealed restriction site or size polymorphism among fish. Maximum sequence divergence between mtDNA haplotypes was low ($p = 0.006$), indicating a maximum divergence time of 300 000 yr. *Coregonus artedi* and *C. hoyi* share mtDNA haplotype arrays indicating a more recent divergence. No unique haplotypes exist to separate *C. artedi* and *C. hoyi* from single collections, although their arrays of haplotypes are significantly different in frequencies. The lack of qualitative differentiation between *C. artedi* and *C. hoyi* noted for mtDNA parallels that previously noted for allozymes and morphology. Introgression between *C. artedi* and *C. hoyi* seems unlikely as the cause for this low amount of differentiation because a 100-base pair insertion haplotype present in Lake Superior and inland lake *C. artedi* is absent in *C. hoyi*.

En utilisant 11 endonucléases messagères de restriction, on a analysé l'ADN mitochondrial (ADNmt) du cisco de lac (*Coregonus artedi*) et du cisco de fumage (*C. hoyi*) capturés dans le lac Supérieur et sept lacs de États du Michigan et de New York afin de déterminer s'il existait un polymorphisme des sites de restriction. Cinq des 11 enzymes ont révélé des sites de restriction ou un polymorphisme de la taille chez les deux espèces. La divergence maximale des séquences entre les haplotypes d'ADNmt était faible ($p = 0{,}006$), ce qui porte à croire que la divergence a eu lieu tout au plus 300 000 ans passés. Les deux espèces partagent des complexes d'haplotypes d'ADNmt qui indiquent une divergence plus récente. Il n'existe aucun haplotype particulier qui permette d'identifer *C. artedi* et *C. hoyi* d'une même collection, bien que la fréquence de leurs complexes d'haplotypes soit nettement différente. L'absence de différences qualitatives entre ces deux espèces pour ce qui est de l'ADNmt correspond à la même situation dans le cas des allozymes et des caractères morphologiques. Il est peu probable que l'introduction entre ces deux espèces de *Coregonus* soit à la source de ce faible niveau de différenciation étant donné qu'un haplotype retrouvé chez *C. artedi* du lac Supérieur et des lacs intérieurs est absent chez *C. hoyi*.

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The coregonine fish of North America and Eurasia have long plagued taxonomists and fisheries biologists in their attempts to identify species and define stocks (Steinmann 1951; Svardson 1970; Nikolsky and Reshetnikov 1970; Behnke 1972; Scott and Crossman 1973; Smith and Todd 1984). The extensive phenotypic plasticity found in coregonines, particularly ciscoes, allows fish of similar genetic background to be phenotypically quite different when reared under varying environmental conditions (Lindsay et al. 1970; Todd et al. 1981) or when sympatric with other species (Lindsey 1981). This plasticity has lead to the description of numerous endemic species and subspecies of ciscoes (Koelz 1929a, 1929b; Hubbs and Lagler 1949, 1958), many of questionable validity (Behnke 1972).

Most Great Lakes ciscoes are refractory to identification by traditional methodologies. Morphometric analysis (Todd and Smith 1980; Todd et al. 1981) is marginally successful at sorting specimens to species by principal components analysis. Analysis of polymorphic allozyme loci (Todd 1981) has proven less useful, as collections of mixed species from single sites appear to represent single, random mating populations.

Analysis of mitochondrial DNA (mtDNA) sequence and size polymorphism has proven useful in identifying species and stocks of fish which are refractory for other means of analysis (see Avise et al. (1987) for a review). For example, heterogeneity among year classes can be assigned to differential contributions of spawning stocks in striped bass (*Morone saxatilis*) (Chapman 1987, 1989), and spawning stocks can also be identified (Chapman 1990) that are not separable by electrophoretic or morphometric analysis (Barlow 1961; Cushing 1975; Grove et al. 1976; Sidell et al. 1980; Rogier et al. 1985). Anadromous populations of ciscoes (*Coregonus artedi*) in the Hudson and James Bay drainages display mtDNA heterogeneity which may represent historical biogeographic events (Bernatchez and Dodson 1990a), and dwarf ciscoes can clearly be shown to be conspecific with normal ciscoes form neighboring lakes which, however, are composed of heterogeneous lineages (Shields et al. 1990).

This paper is an examination of mtDNA variation within and between Lake Superior lake herring (*C. artedi*) and bloaters (*C. hoyi*) and among lake herring from inland lakes in the Lake Superior drainage, the Lake Ontario drainage, and the Susquehanna River drainage to evaluate the efficacy of using mtDNA analysis as a tool for species and stock identifications.

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TABLE 1. Species, collection sites, and number of specimens analyzed for mtDNA restriction fragment length polymorphisms.

Species	Collection site	*N*
Coregonus artedi	Lake Superior, 3 km north of North Entry to Keweenaw Waterway, Houghton Cty., MI	39
	Lake Gogebic, Gogebic Cty., MI	16
	Thousand Island Lake, Gogebic Cty., MI	5
	Ives Lake, Marquette Cty., MI	27
	Pine Lake, Marquette Cty., MI	2
	Otter Lake, Houghton Cty., MI	32
	Hatch Lake, Madison Cty., NY	17
	Lake Skaneateles, Onondaga Cty., NY	8
Coregonus hoyi	Lake Superior, 3 km north of North Entry to Keweenaw Waterway, Houghton Cty., MI	38

Methods

All ciscoes were sampled using gill nets. Species, collection sites, and number of specimens analyzed are listed in Table 1. Fish were identified accoring to Hubbs and Lagler (1958). Fish that were intermediate between key characters were excluded from the analysis. Livers, hearts, and eggs, if present, were dissected from each fish and frozen in liquid nitrogen for transportation to the laboratory. Samples were maintained frozen at −70°C until mtDNA preparation.

mtDNA was purified by differential centrifugation and banding in CsCl/ethidium bromide gradients as described by Lansman et al. (1981) and Maniatis et al. (1982). Closed-circular mtDNA was digested with the following 11 type II restriction endonucleases: Ava I, Bam HI, Bcl I, Bst EII, Eco RI, Hinc II, Hind III, Nde I, Pst I, Pvu II, and Xba I. Fragments produced by restriction enzyme digestion were end-labelled with [α-^{32}P]dATP using T4 DNA polymerase as described by Maniatis et al. (1982). Labelled fragments were separated by molecular weight using agarose gel electrophoresis (Schleif and Wensink 1982). Gels were cast on Gelbond Film (FMC Corp., Rockland, ME) and the percentage of agarose in gels was varied between 0.9 and 1.4% wt/vol to optimize resolution of the fragments produced by each restriction endonuclease. Following electrophoresis, gels were fixed, dried, and then autoradiographed to detect the labelled mtDNA fragments. Hind III digests of bacteriophage λ DNA and a Hinf I digest of pBR322 DNA were used as molecular weight markers. Fragments as small as 200 basepairs (bp) were routinely resolved.

The overall proportion of shared fragments across all digestions (*F*) was calculated for each pair of mtDNA haplotypes using the method of Nei and Li (1979), and sequence divergence values (*P*) were calculated following Upholt (1977). Comparisons of Lake Superior lake herring and bloater stocks were made using a *G*-test of heterogeneity (Zar 1984) on haplotype numbers after pooling haplotypes A′, B′, D, and E′.

Results

Eleven informative restriction endonucleases produced a total of 47 different mtDNA fragments across all species and localities. The recognition sites that delimit these 47 fragments represent a sample of 253 bp or approximately 1.5% of the total mtDNA genome. Table 2 details the arrays of fragments produced by each restriction endonuclease. Total size estimates of mtDNA of 17 000 bp are comparable with those of Bernatchez and Dodson (1990a) for anadromous populations of *C. artedi*. Variation in mtDNA haplotype was of two types, restriction site polymorphism with gains or losses of sites yielding alternative numbers of fragments and size polymorphism within given restriction fragments due to a 100-bp insertion or deletion. Haplotype profiles are given in Table 3 where it may seen that only four of the 11 enzymes produce alternative restriction fragment profiles. The 100-bp insertion/deletion is readily resolved on Hinc II, Hind III, and Ava I digests and is similar to the size polymorphims previously noted for *C. artedi* by Shields et al. (1990), for striped bass (Chapman 1987, 1989), and for numerous other species (cf. Brown and Simpson 1981; Cann and Wilson 1983; Densmore et al. 1985). Restriction site mapping using double digests shows that in all cases the 100-bp insertion/deletion occurs in the same fragment. Double digests with pairs of enzymes that individually do not resolve the 100-bp difference can also resolve this polymorphism. Figure 1 shows a Hinc II digest of mtDNA from fish from several localities which illustrates both restriction site and size polymorphisms. Frequencies of haplotypes are given for each taxon and collection locality in Table 4.

TABLE 2. Fragment sizes (in kilobase pairs) of cisco mtDNA for restriction endonucleases which cut once or more. Fragment pattern types are also given.

Enzyme	Type	Fragment sizes
Ava I	1	3.6, 2.9, 2.4, 2.3, 1.4, 1.1, 1.1, 1.0, 0.7, 0.6
	2	3.6, 2.9, 2.4, 2.4, 1.4, 1.1, 1.1, 1.0, 0.7, 0.6
Bam HI	1	17.1
Bcl I	1	6.8, 6.4, 2.8, 1.1
	2	9.2, 6.8, 1.1
Bst EII	1	16.5, 0.6
Eco RI	1	16.3, 0.8
Hinc II	1	6.6, 3.1, 2.0, 1.6, 1.4, 1.0, 0.7, 0.7
	2	6.6, 3.1, 2.0, 1.7, 1.4, 1.0, 0.7, 0.7
	3	6.6, 2.5, 2.0, 1.6, 1.4, 1.0, 0.7, 0.7, 0.6
Hind III	1	4.3, 3.6, 3.6, 3.3, 2.3
	2	4.3, 3.7, 3.6, 3.3, 2.3
Nde I	1	7.6, 4.8, 3.0, 1.0, 0.7
Pst I	1	12.3, 4.8
Pvu I	1	9.8, 4.9, 1.6, 0.8
Xba I	1	13.4, 3.7
	2	11.6, 3.7, 1.8

TABLE 3. Haplotype profiles of cisco mtDNA. Entries give the fragment pattern types for each enzyme in the order Ava I, Bam HI, Bcl I, Bst EII, Eco RI, Hinc II, Hind III, Nde I, Pst I, Pvu II, and Xba I.

Haplotype	Pattern type										
A	1	1	1	1	1	1	1	1	1	1	1
A′	2	1	1	1	1	2	2	1	1	1	1
B	1	1	2	1	1	1	1	1	1	1	1
B′	2	1	2	1	1	2	2	1	1	1	1
C	1	1	2	1	1	1	1	1	1	1	2
C′	2	1	2	1	1	2	2	1	1	1	2
D	1	1	2	1	1	3	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	2
E′	2	1	1	1	1	2	2	1	1	1	2

mtDNA of *C. artedi* and *C. hoyi* was not highly variable. There was a total of nine different haplotypes across taxa and localities (Table 4). Each mtDNA haplotype is differentiated

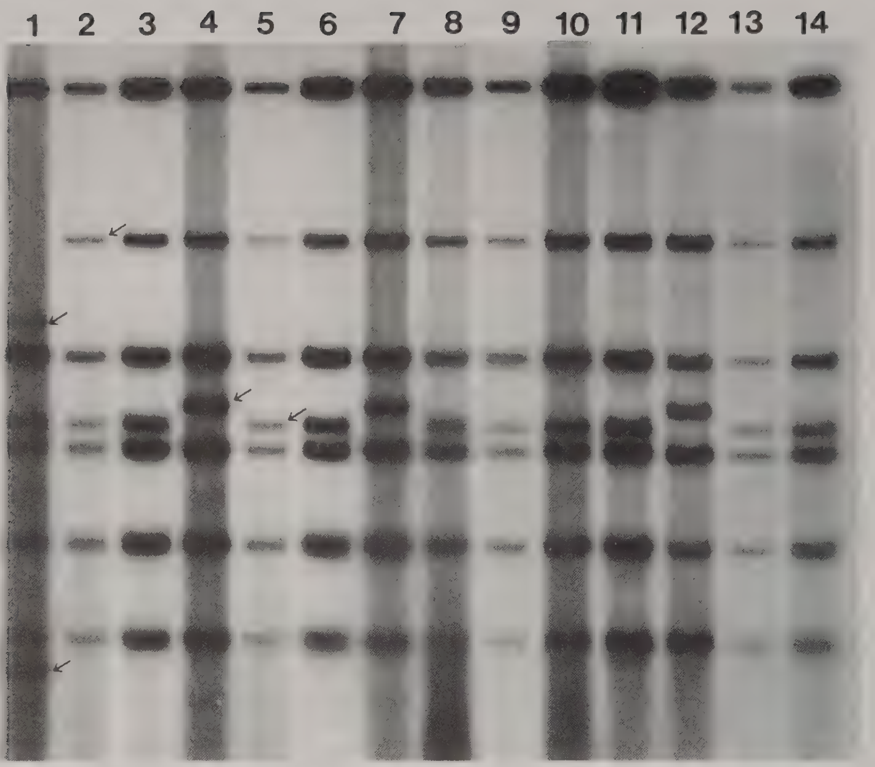

FIG. 1. Hinc II digests of cisco mtDNA. Arrows in lanes 1 and 2 show restriction fragment differences between haplotypes D and B of *C. hoyi*. Arrows in lanes 4 and 5 show the 100-bp insertion difference between *C. artedi* haplotypes A′ and A. Lanes: 1, Lake Superior *C. hoyi* D; 2 and 3, *C. hoyi* B; 4, Lake Superior *C. artedi* A′; 5, Lake Superior *C. artedi* A; 6, Otter Lake *C. artedi* B; 7, Lake Gogebic *C. artedi* A′; 8, Thousand Island Lake *C. artedi* B; 9, Pine Lake *C. artedi* B; 10 and 11, Ives Lake *C. artedi* B; 12, Hatch Lake *C. artedi* B′; 13, Hatch Lake *C. artedi* B; 14, Lake Skaneateles *C. artedi* B.

by the gain and/or loss of one or two restriction sites and/or the presence/absence of a 100-bp insertion/deletion. If one ignores the 100-bp polymorphism, only five mtDNA haplotypes were found. The proportions of shared fragments and estimates of nucleotide sequence divergence among the observed haplotypes are summarized in Table 5.

The arrays of mtDNA haplotypes of Lake Superior *C. artedi* and *C. hoyi* are broadly overlapping (Table 4). The majority of specimens could not be sorted to species based on unique phenotypes. The only exceptions to this are the *C. artedi* haplotypes A′ and B′ which bear the 100-bp insertion which was not detected in *C. hoyi*. While most haplotypes are shared, the distribution of haplotypes differs significantly between the two species ($G = 13.302$, df = 3, $p < 0.005$). These differences in distributions are primarily due to the haplotype C, which is rare in *C. artedi* in our samples, being common in *C. hoyi* and to the absence of the 100-bp insertional haplotypes in *C. hoyi*.

The arrays of haplotypes of lake herring from inland lakes are largely subsets of the array from Lake Superior lake herring except for the two insertion haplotypes C′ and E′ from Lake Gogebic (Table 4). The distribution of haplotypes among the inland lakes is highly heterogeneous, some lakes being fixed for a single haplotype and others having up to four haplotypes.

Discussion

The most striking feature of the mtDNA of Great Lakes *C. artedi* and *C. hoyi* surveyed in this study is their low level of nucleotide sequence divergence. Excluding the size polymorphism, the average divergence among haplotypes was 0.0032 with a maximum divergence between any pair of haplotypes of 0.006. This value is lower than the maximum of 0.010 that Bernatchez and Dodson (1990a) observed between pairs of haplotypes from anadromous *C. artedi*. Nucleotide sequence divergence of mtDNA between ‘‘closely related’’ species of other fish groups is frequently about 10-fold higher as can be seen in the following examples: chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*), 0.034 (Berg and Ferris 1984), European (*Anguilla anguilla*) and American eel (*A. rostrata*), 0.037 (Avise et al. 1986), and nine species of sunfish (*Lepomis*), 0.027–0.0358 (Avise and Saunders 1984). The two isolated groups of lake whitefish (*Coregonus clupeaformis*) present a contrasting picture to other species pairs with an average sequence divergence between lineages of 0.0049 (Bernatchez and Dodson 1990b). In almost all published studies, including that on lake whitefish, species are usually delineated by the possession of unique mtDNA haplotypes even when the degree of nucleotide sequence divergence is low (Avise et al. 1987). Thus the sharing of most of the array mtDNA haplotypes by *C. artedi* and *C. hoyi* may not be consistent with specific status, although the significant difference in fequency of haplotypes suggests some degree of reproductive isolation. Ciscoes from Ives Lake, which have been described as the separate species *C. hubbsi* (Koelz 1929b), also have mtDNA haplotypes like those of other *C. artedi* populations.

TABLE 4. Distributions of mtDNA haplotypes by species and collection sites for ciscoes analyzed. Entries are numbers of specimens and frequencies.

		Haplotype								
Species/site	N	A	A′	B	B′	C	C′	D	E	E′
Coregonus artedii										
Lake Superior	39	19 0.49	3 0.08	12 0.31	2 0.05	2 0.05	— —	1 0.03	— —	— —
Lake Gogebic	16	— —	6 0.38	— —	6 0.38	— —	1 0.06	— —	— —	2 0.13
Thousand Island Lake	5	5 1.00	— —	— —	— —	— —	— —	— —	— —	— —
Ives Lake	27	4 0.15	2 0.07	21 0.78	— —	— —	— —	— —	— —	— —
Pine Lake	2	1 0.50	— —	1 0.50	— —	— —	— —	— —	— —	— —
Otter Lake	32	1 0.03	— —	31 0.97	— —	— —	— —	— —	— —	— —
Hatch Lake	17	1 0.06	— —	12 0.71	3 0.18	— —	— —	1 0.06	— —	— —
Lake Skaneateles	8	— —	— —	8 1.00	— —	— —	— —	— —	— —	
Coregonus hoyi										
Lake Superior	38	11 0.29	— —	15 0.39	— —	11 0.29	— —	— —	1 0.03	— —

TABLE 5. Estimates of sequence divergence, P, top half matrix, and proportion of shared restriction fragments, F, bottom half matrix, between pairs of mtDNA haplotypes of ciscoes.

	A/A′	B/B′	C/C′	D	E/E′
A/A′	—	0.002	0.004	0.004	0.002
B/B′	0.967	—	0.002	0.002	0.004
C/C′	0.935	0.967	—	0.004	0.002
D	0.935	0.967	0.935	—	0.006
E/E′	0.968	0.935	0.968	0.903	—

Numerous authors have attempted to place a maximum time of divergence among mtDNA lineages using a calibration of the mitochondrial molecular clock of 2% sequence divergence per million years (Brown et al. 1979; Shields and Wilson 1987). While some problems apply in using this calibration for fish when it was established for mammalian lineages, it can provide useful relative divergence times and has been widely used for fish (cf. Avise et al. 1987). Application of the clock calibration to the cisco divergence data in this paper would suggest an average divergence time of about 160 000 yr for the cisco lineages with a maximum divergence time of 300 000 yr, far shorter than a radiation beginning at the end of the Pliocene or early Pleistocene as has been suggested by Behnke (1972). The sequence divergence values between the cisco lineages and those of lake whitefish average 0.0451 (T. P. Snyder, unpubl. data) which would correspond to a late Pliocene or early Pleistocene divergence time of about 2 250 000 yr. Interestingly, the sequence divergence values among the cisco haplotypes, which include two nominal species, are of the same magnitude as those observed between clonal groups in the walleye (*Stizostedion vitreum*) (Ward et al. 1989; Billington et al. 1990) and the lake trout (*Salvelinus namaycush*), (Grewe and Hebert 1988; Grewe et al. 1990) and between the Atlantic and western lineages of lake whitefish which appear to be reproductively isolated (Bernatchez and Dodson 1990b).

Identification of stocks of lake herring or bloaters within Lake Superior would appear to present a task about as difficult to accomplish using RFLP analysis of mtDNA as it is with morphometrics (Todd and Smith 1980). While the distribution of mtDNA phenotypes of Lake Superior *C. artedi* was significantly different from that of sympatric collections of *C. hoyi*, individual specimens could not be identified to species, let alone stock, based on mtDNA haplotype. Apart from the 100-bp insertion/deletion polymorphism, there are five different haplotypes (A through E), and 80% of all Lake Superior ciscos sampled were either A or B. The divergence in haplotypes A, B, C, and E can be explained by the gain or loss of single restriction sites for the enzymes Xba I and Bcl I (Fig. 1). The additional haplotype, D, is differentiated by a single Hinc II site and was found in only one Lake Superior *C. artedi*.

A unique feature of some specimens of *C. artedi*, in comparison with *C. hoyi*, is the possession of a 100-bp insertion which was found in 15% of the sampled fish. This size polymorphism has been previously reported in lake herring (Shields et al. 1990) and is similar to that observed in numerous other taxa including the *Morone* spp. (Chapman 1987, 1989, 1990; Mulligan and Chapman 1989), lizards (Densmore et al. 1985; Moritz and Brown 1986), *Rattus* spp. (Brown and Simpson 1981), and man (Cann and Wilson 1983). Densmore et al. (1985) have proposed a mechanism for the rapid accumulation of this type of size polymorphism in mtDNA. In striped bass, size polymorphisms comprise the bulk of observed mtDNA variability and provide useful markers for stock identification in this species which is characterized by strong stock/year classes from relatively few female parents (Chapman 1990). The situation observed in striped bass is considerably different from that observed in lake herring where unexploited populations display a stable age structure and female fecundity is a small fraction of that of striped bass (Bowen et al. 1991). Analysis of specimens from a temporal series and from other localities within Lake Superior will be necessary to fully evaluate the usefulness of RFLP analysis for statistical stock identification of *C. artedi* and *C. hoyi*.

Recent studies of mtDNA from northern cold-water lake species have demonstrated multiple clonal lineages for several species with sequence divergence values consistent with divergence in separate refugia during the Pleistocene glaciations (Bernatchez and Dodson 1990b; Billington et al. 1990; Grewe et al. 1990). Multiple clonal lineages are also present within anadromous *C. artedi* (Bernatchez and Dodson 1990a). No evidence for multiple glacial refugia or clonal lineages exists in the data of this study. Not only are the haplotypes of Lake Superior *C. artedi* and *C. hoyi* widely shared, but inland lake populations of varying degrees of isolation by distance and glacial history share subsets of those found in Lake Superior. The only unique haplotypes found in inland lakes are the C′ and E′ haplotypes of Lake Gogebic. These are both 100-bp insertion haplotypes that may have accumulated rapidly or repeatedly (Densmore et al. 1985).

There is no obvious relationship between the distributions of the mtDNA haplotypes and their distance from Lake Superior or other Great Lakes or their possible glacial history. For example, *C. artedi* sampled from Hatch Lake, in the Susquehanna River drainage, and from Lake Skaneateles, both in upstate New York, have a probable isolation from the Great Lakes of 12 600 yr since the decline of Lake Warren (Calkin and Feenstra 1985) and yet share Lake Superior haplotypes. Lake Gogebic and Thousand Island Lake have had only stream connections with Lake Superior for 11 000 yr and Ives Lake, which has a precipitous stream connection and an age of 8600 yr (Farrand and Drexler 1985), also have Lake Superior haplotypes. Ciscoes are known to be poor upstream dispersers (Svardson 1979). The least isolated lakes in this study, Otter and Pine, with probable continuity with Lake Superior 2200 yr ago (Farrand and Drexler 1985), also share common Lake Superior haplotypes although, especially in the case of Otter Lake, with widely differing frequencies. The study of Shields et al. (1990) on ciscoes in the Mississippi River drainage which was based on three restriction enzymes, which are the same as those most polymorphic in this study, also shows clonal types in common with Lake Superior haplotypes (their clonal types A, B, C, and D would appear to correspond to our haplotypes B, C, A, and E, respectively). The distribution of mtDNA phenotypes within inland lakes is most likely the result of genetic drift or founder effect with each inland lake population being derived from the more highly polymorphic pool which restablished itself in the Great Lakes sensu latu following the retreat of the last glacial ice sheet.

It is unclear if the ciscoes in Lake Superior represent multiple, reproductively isolated species. The statistically heterogeneous distributions of haplotypes found in sympatric collections clearly demonstrate that some degree of reproductive isolation occurs between *C. artedi* and *C. hoyi*, probably based on behavioral or temporal mechanisms, as they are completely interfertile (Garside and Christie 1962). On the other hand the species are exceedingly closely allied. They share virtually identical complements and frequencies of alleles at allozymic loci (Todd 1981). The sharing of complements of mtDNA haplotypes which indicates a common pool of maternal lineages is consistent with ecological or behavioral differentation within the post-Wisconsin glaciation Great Lakes within the last 14 500 yr as has been suggested by Booke (1968) but not with differentiation in earlier glacial episodes or in separate glacial refugia. This lack of differentiaton between taxa is unlikely to be due to hybridization between overexploited species as has been suggested by Smith (1964), as virtually all Lake Superior lake herring mtDNA haplotypes are found in one or more inland lakes and the 100-bp insertion haplotype present in lake herring is absent in the bloaters which were not depressed in numbers to a significant degree.

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Effects of Grazer Community Composition and Fish on Algal Dynamics

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We examined the effects of grazer community composition and fish on phytoplankton abundance by manipulating zooplankton community structure and the intensity of planktivory in a factorial experiment. Enclosures (1700-L bags) were treated with fish (present/absent) and two grazer communities (one a large-bodied community dominated by *Daphnia* and the other a small-bodied community dominated by *Ceriodaphnia*) in a 2 × 2 factorial design. We sampled zooplankton and algae every 4–8 d during the 5-wk experiment. Algal biovolume, chlorophyll a, total particulates, and light extinction were all significantly higher in the presence of fish. Further, the effect of fish on algal standing crop did not depend on which grazer assemblage was initially present. Fish enhanced algal standing crop to the same degree in both *Daphnia* and *Ceriodaphnia* treatments. We discuss these results in light of patterns reported in the literature, and the nature of size-structured interactions among fish, zooplankton, and algae.

On a étudié les incidences de la composition de la communauté de brouteurs et de poissons sur l'abondance dy phytoplancton en manipulant la structure de la communauté zooplanctonique et l'intensité de la planctivorie lors d'une expérience de factorielle. Le plan factoriel était basé sur toutes les combinaisons des deux facteurs poisson (absent ou présent) et communautés de brouteurs (une communauté de gros brouteurs dominée par *Daphnia* et une communauté de petits brouteurs dominée par Ceriodaphnia) gardés dans des enclos de 1 700 L. Le zooplancton et les algues ont été échantillonnées à tous les 4 à 8 jours au cours de l'expérience de 5 sem. Le biovolume d'algues, le teneur en chlorophylle a, la teneur en matières particulaires et l'atténuation de la lumière étaient nettement plus élèvés lorsque des poissons étaient présents. De plus, l'incidence des poissons sur la biomasse des algues ne dépendait pas de l'assemblage de brouteurs initialement présents. La présence de poissons a mené à la mêne augmentation de la biomasse algale lorsque *Daphnia* et *Ceriodaphnia* étaient présents. On étudie les résultats à la lumière des régimes relevés dans des études publiées, et de la nature des interactions, structurées selon la taille, entre les poissons, le zooplancton et les algues.

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The cascading effects of fish on lower trophic levels continues to be a strong research focus in freshwater pelagic ecosystems (Carpenter and Kitchell 1992). Of particular interest is the control of phytoplankton standing crop through the alteration of food web structure, i.e. biomanipulation (reviews in Benndorf 1987 and Gophen 1990). Piscivorous fish are often stocked into lakes in an effort to reduce the death rate planktivores impose on herbivorous zooplankton, either by decreasing planktivore numbers (e.g. Scavia et al. 1986; Benndorf et al. 1988) or by inducing planktivores to shift habitats (e.g. Turner and Mittelbach 1990), thereby allowing grazer standing crop to increase. Leibold (1989) reviewed 32 controlled field experiments in which planktivory was manipulated (table 4 in Leibold 1989). The results of these experiments show the following general pattern. In lakes dominated by large-bodied grazers of the genus *Daphnia*, manipulations of planktivory significantly affected algal standing crop in 19 of 22 studies. However, in lakes containing only small-bodied cladocerans, planktivore manipulations affected algal standing crop in only 3 of 10 experiments. Thus, for this set of experiments the outcome of manipulating planktivory on algal standing crop was strongly dependent on grazer community composition (Leibold 1989).

Several authors have noted the above pattern and hypothesized that the presence of *Daphnia* is a critical factor in determining the strength of trophic interactions in lakes (Carpenter and Kitchell 1984; McQueen et al. 1986; Leibold 1989). However, no single experiment has tested this hypothesis by independently manipulating planktivory and grazer community composition in the same lake. The possibility exists that grazer community composition per se is not the important factor, but that some correlated variable (i.e. lake trophic status, phytoplankton community composition) is confounded with grazer community composition and is responsible for the observed pattern. Here we present the results of a field experiment in which we explicitly examine whether the effects of fish on phytoplankton depend on initial grazer community composition.

Methods

Planktivorous fish abundance (fish/no fish) and grazer community composition (*Daphnia* or *Ceriodaphnia*) were manipulated in a 2 × 2 factorial design. In this design, any effect of fish manipulations on algal standing crop that depends on initial grazer community composition would be expressed as a significant fish abundance by grazer community composition interaction term. Based on the field pattern, we expect that fish will enhance algal standing crop to a greater extent in the presence of *Daphnia* than in the presence of *Ceriodaphnia*.

We placed twelve 1700-L clear polyhexene enclosures (1.1 m in diameter by 1.8 m deep, sealed off from the sediments at the bottom) into one of the 18 experimental ponds (30 m in diameter and 1.8 m deep) at the Kellogg Biological Station of Michigan State University. The experimental ponds contain a diverse assemblage of macroinvertebrates, including a cladoceran-dominated zooplankton community similar to those found in local lakes (Geedey 1990; Turner and Mittelbach 1990). The pond used for this experiment contained approximately 4000 bluegill (*Lepomis macrochirus*), some 90% of which were young-of-the-year. Total phosphorus concentrations among the ponds average 13 µg/L (Geedey 1990), and the ponds would thus be classified as mesotrophic (Wetzel 1983). Water temperature in the experimental pond ranged from 24.5 to 20.0°C during the course of this experiment.

The 12 enclosures were filled on 17 August with pond water filtered through a 153-µm-mesh net to remove all macrozooplankton. We then randomly assigned three enclosures to each of the four treatments (*Daphnia, Ceriodaphnia, Daphnia* + fish, *Ceriodaphnia* + fish). *Ceriodaphnia reticulata* were collected from an adjacent pond by taking repeated vertical tows with a 153-µm-mest net and were stocked into half of the bags at a mean density (± 1 SE) of 71.1 ± 3.7 µg/L (dry mass). We collected *Daphnia pulicaria* from nearby Lawrence Lake and attempted to stock them into the bags at a biomass equal to that in the *Ceriodaphnia* treatment, although the actual density stocked was somewhat lower; (mean (± 1 SE) = 54.5 ± 4.9 µg/L). The zooplankton were given several days to become established, and on 23 August, two small bluegills (mean standard length (± 1 SE) = 42.2 ± 2.4 mm, N = 12 fish) were added to three of the bags with *Daphnia* and three bags with *Ceriodaphnia*. This fish density (2.1 bluegills/m^2 or 2.69 g (wet mass (m/^{3}) is lower than that in the pond itself (approximately 5.6 bluegills/m^2 or 10 g/m^3) and is similar to the bluegill density in local lakes (Mittelbach 1988).

To monitor zooplankton and algal responses to the manipulations, we sampled zooplankton and algae every 4 d from 24 August through 5 September and then every 8 d through 21 September. Zooplankton were sampled at night with a 19-L Schindler trap fitted with a 60-µm-mesh net. One sample was taken from 1 m deep in each bag, and the zooplankton were preserved immediately in a 5% solution of cold sugar formalin. Abundant species were counted by dividing the sample in half; for rarer species the entire sample was enumerated. For each sample, the body lengths of at least 50 individuals of each species, or all individuals if <50 were present in the sample, were measured. Body length distributions, combined with density estimates, were used to calculate grazer biomasses, using the length–weight regressions of Dumont et al. (1975), Bottrell et al. (1976), and Culver et al. (1985). Total grazer biomass includes herbivorous rotifers, cladocerans, and copepods. Cladocerans averaged 76% of the total grazer biomass over the course of the experiment. Since cladoceran mass-specific filtering rates are over an order of magnitude higher than copepod filtering rates (Knoechel and Holtby 1986), we calculated the biomass of these important grazers separately. In addition, we calculated the cladoceran community grazing rates (equal to per capita algal mortality) from the regression equations of Mourelatos and Lacroix (1990). We used the grazing rates measured by Mourelatos and Lacroix because their study lake is similar to our pond in its physical and chemical properties and in the algal assemblage it contains. In addition, they measured grazing rates on labeled *Chlorella*, a small chlorophyte that was common in our enclosures. The regression equations predict individual filtering rates as a function of body length, species identity (*Daphnia, Ceriodaphnia*, or *Bosmina*), and water temperature. The appropriate individual filtering rates were multiplied by our measurements of the density of each size class, producing an estimate of the overall cladoceran community grazing rate.

Phytoplankton samples for in vivo fluorometric analysis were collected at midday with an integrated tube sampler (3.8 cm in diameter, 0–0.7 m depth). Fluorometric analyses of chlorophyll concentrations were performed after the methods of Kiefer (1973) and Heaney (1978). We analyzed both whole-water samples, as well as samples filtered through a 16-µm sieve, so as to estimate the ''edible'' fraction of the algal standing crop (Burns 1968). We determined the relationship between fluorescence values and chlorophyll *a* concentrations by performing acetone extractions and spectrophotometric analyses on a subset of the samples, and we then used this relationship to convert all fluorometric values into chlorophyll *a* concentrations. In addition, direct cell counts were done on 320-mL samples gathered on the final sampling date and fixed with Lugol's solution. These were later counted by allowing a subsample to settle 24 h in a 30-mL sedimentation chamber and then counting the cells in 15–25 fields of view at 250× (1.1–1.9% of the sample) using a Zeiss inverted microscope. We measured approximately 20 cells of each taxon at 400× and used standard geometric formulae, along with cell counts, to calculate algal biovolume.

We measured water transparency and total particulates on three occasions during the latter half of the experiment, after algal dynamics had stabilized. Water transparency was measured with a Li-Cor photometer and the extinction coefficient calculated as in Wetzel and Likens (1979). We calculated the extinction coefficient for the surface to 0.5-m interval separately from the 0.5- to the 1.0-m interval and used the mean of the two values in all analyses. Total particulates (less than 60 µm) were measured by prefiltering 3–3.6 L of water through a 60-µm sieve and onto preashed glass fiber filters (Whatman GF/F), drying at 80°C for 48 h, and weighing. Particulate organic matter (POM) was measured by weighing the filters after ashing them at 500°C for 30 min.

We analyzed the data with a two-way repeated-measurements (split-plot) analysis of variance (ANOVA) (Winer 1971; Gill 1978) using the following model: $Y_{ijkl} = U + \alpha_i + \beta_j + (\alpha\beta)_{ij} + C_{(ij)k} + T_l + (\alpha T)_{il} + (\beta T)_{jl} + (\alpha\beta T)_{ijl} + E_{ijkl}$, where the terms are overall mean (U), effect of fish presence/absence (α), effect of grazer identity (β), fish × grazer interaction ($\alpha\beta$), effects of enclosures within treatments (C), time effect (T), time × fish interaction (αT), time × grazer interaction (βT), time × fish × grazer interaction ($\alpha\beta T$), and residual error (E). The enclosure term (C) serves as error$_1$ to test treatment effects; residual error (E) represents error$_2$ to test the effects of time and time × treatment interactions (Gill 1986). For main effects, repeated-measurements ANOVA is equivalent to taking a mean of all dates for each bag, with each bag thus generating one observation, and then using a conventional ANOVA to test for treatment effects. In a repeated-measurements ANOVA the significance tests of time × treatment interactions assume a homogenous correlation structure among dates. We tested this assumption and used the Geisser–Greenhouse correction (Geisser and Greenhouse 1958) when it was not supported ($P <$ 0.20). Visual inspection indicated that variance tended to increase with the mean; therefore, all data were log transformed. All analyses were done using the Statistical Analysis System (SAS Institute Inc. 1985).

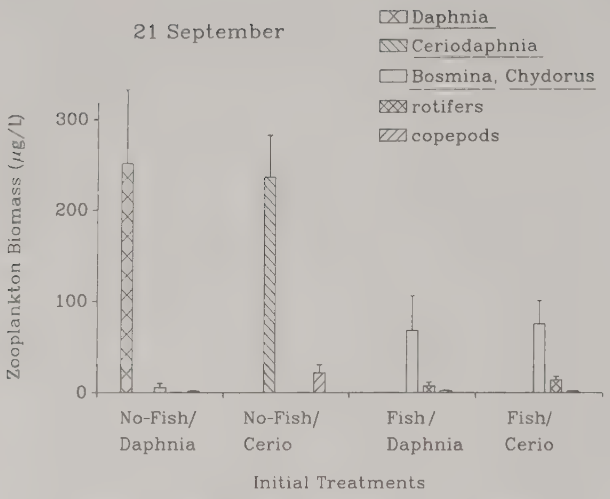

FIG. 1. Biomass of herbivorous zooplankton in each treatment at the end of the experiment. Shown are the mean values for the three enclosures within a treatment, except for the no-fish/*Ceriodaphnia* treatment which includes two enclosures; error bars represent 1 SE. "*Daphnia*" includes *Daphnia pulicaria* and a few *Daphnia galeata mendotae*; "*Bosmina, Chydorus*" includes a few *Alona* and *Scapholeberis*; "copepods" includes calanoids, cyclopoids, and nauplii.

Unfortunately, on our first sample date (24 August), we noted that an enclosure in the no-fish/*Ceriodaphnia* treatment lacked *Ceriodaphnia* whereas the other two enclosures in the treatment contained 131 and 73 *Ceriodaphnia*/L. *Ceriodaphnia* were never detected in this one enclosure whereas they thrived in the other two enclosures in the treatment. Since the grazer community was an imposed treatment, and we failed for unknown reasons to successfully impose it in this one enclosure, we removed this enclosure from the analyses.

Results

Fish Survivorship and Growth

All bluegills were successfully recovered from the enclosures at the end of the experiment and were measured and wet masses calculated from length–weight relations (Osenberg et al. 1988). The fish grew from a mean mass of 2.31 g to a final mass of 2.70 g, for an average increase of 17%. Grazer treatment had no effect on fish growth (*t*-test, $P > 0.25$).

Zooplankton Dynamics

In the absence of fish, *Daphnia* and *Ceriodaphnia* dominated their respective zooplankton communities (Fig. 1), comprising greater than 97% of the Cladocera biomass and greater than 92% of the total grazer biomass, in their respective no-fish treatments by the end of the experiment. However, in the presence of fish, both *Daphnia* and *Ceriodaphnia* were quickly eliminated (by 28 August) and were replaced by the very small cladocerans *Bosmina longirostris* and *Chydorus sphaericus*, as well as various rotifers (Fig. 1). Thus, the impact of fish was to shift both the *Daphnia* and *Ceriodaphnia* treatments to a community dominated by very small cladocerans (Fig. 1). Cladoceran body size was significantly smaller in the fish treatments ($P < 0.01$) (Table 1; Fig. 2a), and the effect of fish on cladoceran body size depended on grazer treatment, i.e. the fish × grazer interaction term was significant ($P < 0.01$). In addition, the grazer treatment had a significant effect on cladoceran body size ($P < 0.01$), and this effect is consistent over the course of the experiment ($P > 0.25$), whereas the fish effect on cladoceran body size appears to become more pronounced during the experiment ($P = 0.02$, time × fish interaction). As expected, the overall effect of fish was to strongly depress Cladocera and total grazer biomass ($P < 0.01$) (Table 1; Fig. 2b). Note that while we attempted to stock *Daphnia* at a biomass similar to that of *Ceriodaphnia*, the initial survivorship of *Daphnia* was poor and *Daphnia* started at a low density (Fig. 2b). *Daphnia* increased from their initial low numbers throughout the experiment in the no-fish treatment whereas *Ceriodaphnia* maintained a relatively constant biomass in the absence of fish (significant time × fish × grazer interaction in Cladocera biomass, Table 1). Cladoceran biomass in the presence of fish was similar regardless of the initial zooplankton community (Fig. 2b).

TABLE 1. Two-way repeated-measures ANOVA's of zooplankton community responses to fish and grazer manipulations. Probability values estimated by Geisser–Greenhouse correction denoted by an asterisk.

Source of variation	ss	df	ms	*F*	*P*
Cladoceran body size					
Fish density	28.06	1	28.06	112.6	<0.01
Grazer identity	6.60	1	6.60	26.5	<0.01
Fish × grazer	5.58	1	5.58	22.4	<0.01
$Error_1$	1.74	7	0.25		
Time	2.00	5	0.40	2.82	0.09*
Time × fish	3.58	5	0.72	5.05	0.02*
Time × grazer	0.22	5	0.04	0.31	0.73*
Time × fish × grazer	0.39	5	0.08	0.55	0.58*
$Error_2$	4.96	35	0.14		
Total grazer biomass					
Fish density	57.77	1	57.77	234.1	<0.01
Grazer identity	1.84	1	1.84	7.45	0.03
Fish × grazer	1.16	1	1.16	4.72	0.07
$Error_1$	1.73	7	0.25		
Time	8.83	5	1.77	6.42	0.01*
Time × fish	7.43	5	1.49	5.40	0.02*
Time × grazer	8.45	5	1.69	6.14	0.02*
Time × fish × grazer	3.19	5	0.64	2.32	0.14*
$Error_2$	9.64	35	0.27		
Cladocera biomass					
Fish density	133.05	1	133.05	87.94	<0.01
Grazer identity	0.01	1	0.01	0.00	0.97
Fish × grazer	22.67	1	22.67	14.98	<0.01
$Error_1$	10.59	7	1.51		
Time	64.23	5	12.84	10.88	<0.01
Time × fish	13.41	5	2.68	2.27	0.07
Time × grazer	7.71	5	1.54	1.31	0.28
Time × fish × grazer	17.56	5	3.51	2.97	0.02
$Error_2$	41.33	35	1.18		
Cladoceran community filtering rates					
Fish density	217.04	1	217.04	182.57	<0.01
Grazer identity	2.02	1	2.02	1.70	0.23
Fish × grazer	40.72	1	40.72	34.25	<0.01
$Error_1$	8.32	7	1.19		
Time	55.78	5	11.15	7.05	<0.01
Time × fish	13.48	5	2.69	1.70	0.15
Time × grazer	9.69	5	1.94	1.22	0.32
Time × fish × grazer	16.52	5	3.30	2.09	0.09
$Error_2$	55.40	35	1.58		

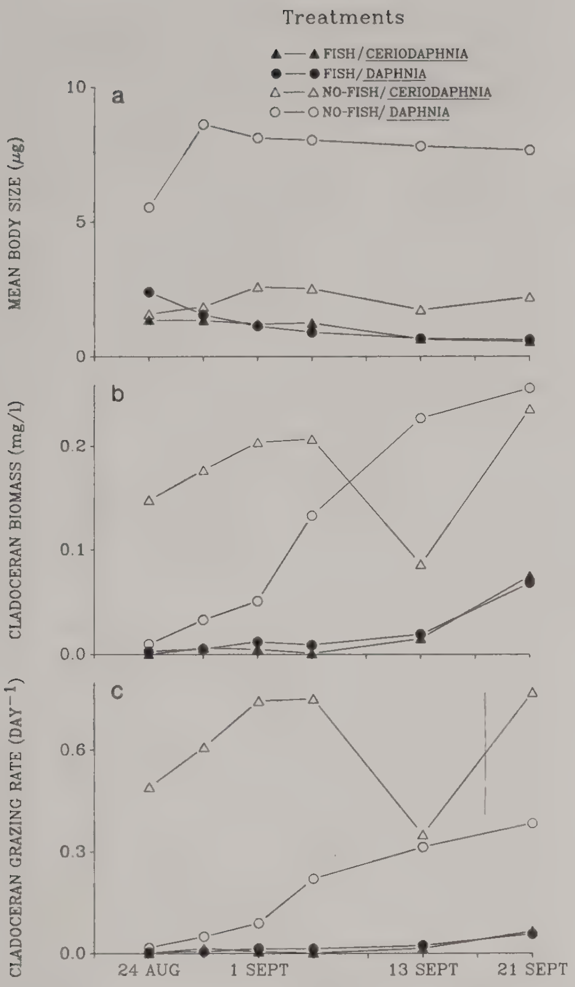

FIG. 2. Cladoceran dynamics over the course of the experiment. Symbols refer to the initial experimental treatments. Fish eliminated both *Daphnia* and *Ceriodaphnia* from the enclosures; thus, enclosures with fish were dominated by *Bosmina* and *Chydorus*. (a) Mean body size of cladocerans in each treatment; (b) mean cladoceran biomass in each treatment; (c) mean cladoceran daily grazing rate.

Cladoceran community grazing rates were higher in the *Ceriodaphnia* treatment than in the *Daphnia* treatment in the absence of fish ($P < 0.01$, fish × grazer interaction on cladoceran community grazing rates) (Table 1; Fig. 2c). This is partially because *Ceriodaphnia* maintained a higher biomass than did *Daphnia* in the absence of fish and partially because *Ceriodaphnia* have a higher mass-specific filtering rate than do *Daphnia* on small food particles (Chow-Fraser and Knoechel 1985; Knoechel and Holtby 1986; Mourelatos and Lacroix 1990). The strong effect of fish on cladoceran biomass and size resulted in much lower cladoceran grazing rates in the presence of fish ($P < 0.01$, fish density effect) (Table 1; Fig. 2c). Over the final four sample dates, cladoceran community grazing rates averaged 0.65/d in the no-fish/*Ceriodaphnia* treatment and 0.25/d in the no-fish/*Daphnia* treatment, a 2.6-fold difference. However, when grazer treatments are combined, cladoceran community grazing rates averaged 0.45/d in the absence of fish and only 0.024/d in the presence of fish, a 19-fold difference.

TABLE 2. Two-way repeated-measures ANOVA's of treatment effects on various estimates of algal standing crop.

Source of variation	ss	df	ms	*F*	*P*
Chlorophyll a, entire experiment					
Fish density	9.65	1	9.65	66.72	<0.01
Grazer identity	1.94	1	1.94	13.43	<0.01
Fish × grazer	0.08	1	0.08	0.56	0.48
Error$_1$	1.01	7	0.14		
Time	12.39	5	2.47	8.75	<0.01
Time × fish	8.34	5	1.67	5.89	<0.01
Time × grazer	5.41	5	1.08	3.82	<0.01
Time × fish × grazer	9.09	5	1.82	6.42	<0.01
Error$_2$	9.92	35	0.28		
Chlorophyll a, final four sample dates					
Fish density	15.80	1	15.80	93.00	<0.01
Grazer identity	0.51	1	0.51	2.99	0.13
Fish × grazer	0.13	1	0.13	0.76	0.41
Error$_1$	1.19	7	0.17		
Time	2.79	3	0.93	2.44	0.09
Time × fish	2.05	3	0.68	1.79	0.18
Time × grazer	4.31	3	1.44	3.76	0.03
Time × fish × grazer	6.86	3	2.29	6.00	<0.01
Error$_2$	8.01	21	0.38		
Total particulates					
Fish density	2.72	1	2.82	18.55	<0.01
Grazer identity	0.04	1	0.04	0.25	0.63
Fish × grazer	0.12	1	0.12	0.77	0.41
Error$_1$	1.07	7	0.15		
Time	0.02	2	0.01	0.16	0.85
Time × fish	0.38	2	0.19	3.62	0.05
Time × grazer	0.69	2	0.35	6.50	0.01
Time × fish × grazer	0.11	2	0.05	1.03	0.38
Error$_2$	0.75	14	0.05		
Light extinction					
Fish density	0.241	1	0.241	27.90	<0.01
Grazer identity	0.001	1	0.001	0.12	0.74
Fish × grazer	0.056	1	0.056	6.55	0.04
Error$_1$	0.060	7	0.009		
Time	0.234	2	0.117	5.70	0.02
Time × fish	0.037	2	0.018	0.89	0.43
Time × grazer	0.004	2	0.002	0.09	0.91
Time × fish × grazer	0.081	2	0.040	1.97	0.18
Error$_2$	0.288	14	0.020		

Phytoplankton Dynamics

Chlorophyll *a* concentrations were significantly higher in the presence of fish for both *Ceriodaphnia* and *Daphnia* treatments ($P < 0.01$) (Table 2; Fig. 3a). In the no-fish/*Ceriodaphnia* treatment, there was an initial fluctuation in chlorophyll *a* concentration (Fig. 3a), after which the four treatments settled down to a very consistent pattern over the final four sample dates (a 23-d period). Because of these initial dynamics, chlorophyll *a* over all six dates was significantly higher in the *Ceriodaphnia* treatment ($P < 0.01$). However, grazer community composition had no significant effect on chlorophyll *a* over the final four dates ($P = 0.13$). Further, the effect of fish on chlorophyll *a* concentrations did not depend on which grazer community was initially present, i.e. the fish × grazer interaction term was not significant ($P > 0.4$).

Total particulates, like chlorophyll *a*, were higher in the presence of fish ($P < 0.01$) (Table 2; Fig. 3b), but initial grazer

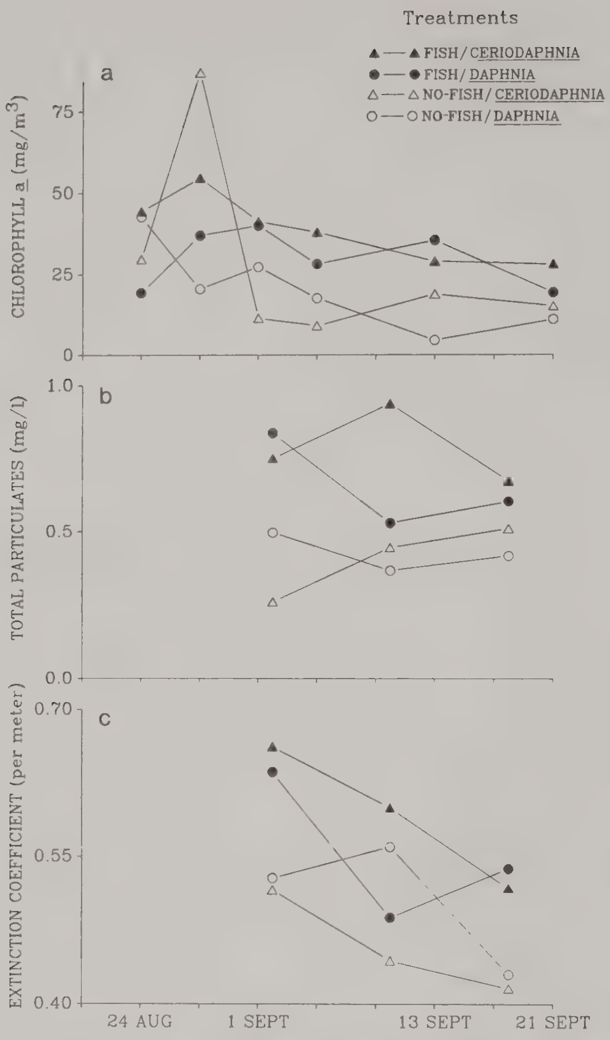

FIG. 3. Various measures of algal dynamics over the course of the experiment; symbols as in Fig. 2. (a) Mean chlorophyll *a* concentrations in each treatment; (b) mean mass of total particulate matter <60 μm suspended in the water column; (c) mean rate of light extinction in each treatment.

identity had no effect on total particulates, and the effect of fish on total particulates did not differ in the *Ceriodaphnia* or *Daphnia* treatments ($P > 0.05$). On 1 September, POM (± 1 SE) composed 58.3 + 5.9% ($N = 12$) of the total particulate mass. A two-way ANOVA performed on the 1 September POM data shows a fish effect ($P = 0.02$) but no effect of initial grazer treatment ($P = 0.32$) and no fish × grazer interaction ($P = 0.32$). Over the final two sample dates, POM composed greater than 92% of the total particulate mass in every enclosure, so POM results mirror those of total particulates. Light extinction was also significantly increased by fish ($P < 0.01$) (Table 2; Fig. 3c) but was not affected by the grazer treatment ($P > 0.05$). However, there was a significant fish × grazer interaction ($P = 0.04$) due to the differential response of the no-fish/*Daphnia* and fish/*Daphnia* treatments on 10 September.

TABLE 3. Phytoplankton biovolume on 21 September. Edible algae are assumed to be those cells <20 μm GALD and include cryptomonads, chlorophytes, diatoms, and other chrysophytes. Inedible algae were mostly colonial chlorophytes (*Gloeocystis*).

Treatment	Algal biovolume (10^3 μm³/mL)	
	Edible	Inedible
No-fish/*Daphnia*	143.0 ± 86.9	0.9 ± 0.6
No-fish/*Ceriodephnia*	78.6 ± 37.6	13.0 ± 5.2
Fish/*Daphnia*	302.8 ± 41.9	<0.1
Fish/*Ceriodaphnia*	250.3 ± 45.0	<0.1

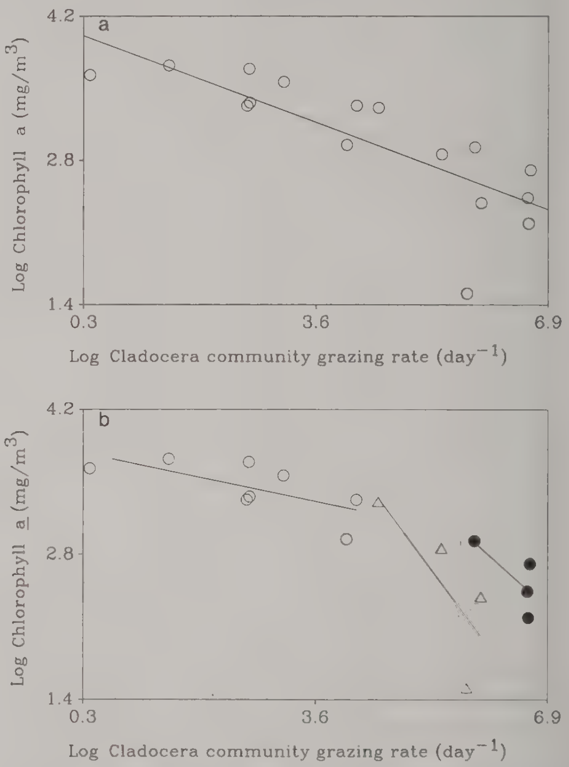

FIG. 4. Relationship between Cladocera community grazing rates and chlorophyll *a* concentrations. $N = 16$ points; each point represents the mean value for a unique treatment date combination ($N = 4$ treatments, 4 dates). (a) Overall relationship between the natural logarithm of Cladocera community grazing rates (CGR) and chlorophyll *a* (Chl *a*) ($\log \text{Chl } a = -0.25 \cdot \log \text{CGR} + 4.09$; $r^2 = 0.64$, $P < 0.01$); (b) influence of zooplankton community on this relationship. Open circles represent enclosures dominated by *Bosmina*/*Chydorus* (with fish), open triangles represent enclosures dominated by *Daphnia* (no fish), and closed circles represent enclosures dominated by *Ceriodaphnia* (no fish).

Algal counts from the final sample date are consistent with the above pattern. Algal biovolume on 21 September is significantly higher in the presence of fish ($P = 0.03$) but shows no effect of initial grazer community or any fish × grazer interaction ($P > 0.25$). The algal community was dominated by small, highly edible chlorophytes and cryptomonads in all treatments, with cells >20 μm greatest axial linear dimension (GALD) comprising less than 15% of the total algal biovolume in each treatment. Thus, all measures of algal response (chlorophyll *a*, total particulates, light extinction, biovolume) show a strong, positive effect of fish but little or no long-term effect of initial grazer community (i.e. *Daphnia* or *Ceriodaphnia*).

One of the fundamental questions of biomanipulation is how phytoplankton standing stocks respond to variation in zooplankton abundance or grazing intensity and whether the relationship between phytoplankton abundance and zooplankton grazing differs for different zooplankton communities. To address this issue, we regressed the natural logarithm of phytoplankton standing stock (as measured by chlorophyll *a*) as a function of log cladoceran grazing rate over the last four sampling dates in the experiment (when algal dynamics had stabilized). As expected, chlorophyll *a* was significantly, negatively related to cladoceran grazing rate (Fig. 4a), and cladoceran grazing rate explains a substantial portion of the variation in chlorophyll *a* ($r^2 = 0.64$). The overall relationship between chlorophyll *a* and cladoceran grazing rate (Fig. 4a) can be further decomposed into the three functional cladoceran communities found through most of the experiment (i.e. *Daphnia* without fish, *Ceriodaphnia* without fish, *Bosmina*/*Chydorus* with fish). Figure 4b suggests that the relationship between algal standing stock and zooplankton grazing rate may differ among these three communities ($P = 0.08$, heterogeneity of slopes test), with the *Bosmina*/*Chydorus* community having a shallower slope. However, this result should be regarded as tentative, since the regressions for *Daphnia* and *Ceriodaphnia* are based on only a very limited number of data points.

Discussion

Our various measures of phytoplankton standing crop show a consistent pattern. First, the presence of fish enhanced algal standing crop and this effect did not depend on whether *Daphnia* or *Ceriodaphnia* were initially present. Second, in the absence of fish, *Daphnia* and *Ceriodaphnia* maintained algal abundances at similar levels. The first result is at odds with the pattern gleaned from Leibold's (1989) review of the literature, i.e. that the positive effect of fish on algae is much more common in *Daphnia*-dominated communities than in communities composed of small-bodied cladocerans. We note that this difference is not due to our selection of *Ceriodaphnia* as the representative small-bodied cladoceran. Of the 10 studies in Leibold's review involving small-bodied cladoceran's, four were *Ceriodaphnia*-dominated communities, yet the effect of fish on algae did not depend on whether *Ceriodaphnia* was the dominant grazer ($P > 0.3$, $\chi^2 = 0.08$, df = 1). The second result also differs from a number of studies showing that *Daphnia* depress algal standing stocks more than do small-bodied cladocerans (e.g. Pace 1984; Schoenberg and Carlson 1984; Vanni 1986; Elser et al. 1987; Dawidowicz 1990; Elser et al. 1990). We discuss both results below and suggest how the size structure of the phytoplankton community and the size-selective nature of fish foraging may help reconcile these seemingly conflicting results.

It has been shown that large *Daphnia* are able to feed on a broader range of algal sizes and to impose a higher mortality rate on large algae than do small-bodied cladocerans (Burns 1968; Gliwicz 1980; McCauley and Downing 1985). However, there is no evidence demonstrating that *Daphnia* feeding on small, edible algae have a higher size-specific feeding ability than do small-bodied cladocerans. In fact, there is some evidence for the opposite response (e.g. DeMott 1982). Thus, we would expect to find the greatest difference between the impact of large *Daphnia* and small-bodied cladocerans when the phytoplankton contains both large and small forms and the least difference when the phytoplankton is composed only of small species.

Most studies showing that *Daphnia* depressed chlorophyll *a* more than did small-bodied cladocerans have used phytoplankton communities containing both large and small cells (Pace 1984; Schoenberg and Carlson 1984; Vanni 1986; Elser et al. 1987; Dawidowicz 1990; Elser et al. 1990). In contrast, almost all the algae in our experiment were small and highly edible, and there was no difference in the effect of *Daphnia* and *Ceriodaphnia* on algal standing stock. Thus, the differential effects of large-, and small-bodied Cladocera on algae and the difference between our results and those of other field experiments appear to depend on the size-structure of the algal population. We did find that large cells were more common in the no-fish/*Ceriodaphnia* treatment than in the no-fish/*Daphnia* treatment by the end of the experiment (Table 3); however, these large cells comprised only 14% of the total algal biovolume in the no-fish/*Ceriodaphnia* treatment. Had we allowed the experiment to run longer, or had large phytoplankton been more common initially, it is possible that the two grazer treatments would have produced differential effects on phytoplankton dynamics.

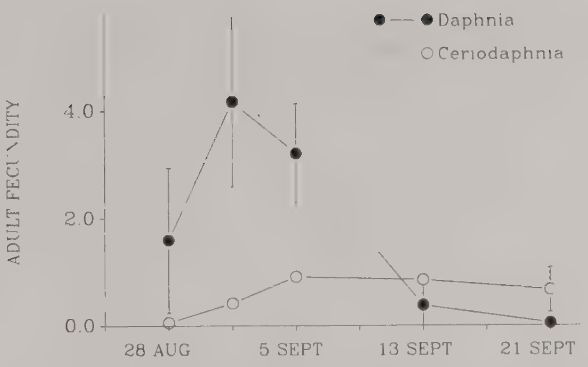

FIG. 5. Fecundity of cladocerans in the absence of fish. Closed circles represent the mean number of eggs per adult *Daphnia* in fishless enclosures ($N = 3$) and open circles represent the mean number of eggs per adult *Ceriodaphnia* in fishless enclosures ($N = 2$); error bars represent 1 SE.

The high community grazing rates of *Ceriodaphnia* relative to *Daphnia* in our experiment demonstrate that zooplankton communities composed of small-bodied cladocerans do not necessarily have lower grazing rates than *Daphnia*-dominated communities. In fact, given the overall relationship between grazer body size and individual grazing rates of Peters and Downing (1984), and holding total grazer biomass constant, community grazing rates actually increase with decreasing body size (as noted by Sterner 1989). However, there is strong empirical evidence that zooplankton communities dominated by *Daphnia* commonly have higher grazing rates than small-bodied zooplankton communities (Sterner 1989). Thus, *Daphnia*-dominated communities have high grazing rates because they reach a higher biomass than do small-bodied cladoceran communities. In our experiment, *Daphnia* biomass generally did not exceed *Ceriodaphnia* biomass. While one might argue that *Daphnia* densities have not yet reached carrying capacity in our experiment, this does not appear to be the case. *Ceriodaphnia* sustained a mean fecundity of at least 0.67 eggs/adult over the latter half of the experiment, while adult *Daphnia* fecundity fell from 4.17 eggs/adult on 1 September to 0.05 eggs/adult on 21 September (Fig. 5). Therefore, it appears unlikely that *Daphnia* would have reached a higher biomass than *Ceriodaphnia*. The fact that *Daphnia* and *Ceriodaphnia* achieved similar final biomasses in our experiment, coupled with the small size structure of the phytoplankton community, accounts for the result that *Daphnia* and *Ceriodaphnia* maintained algal abundances at similar levels in the absence of fish. Thus, while the clear and consistent effect of phanktivorous fish is to shift zooplankton communities to smaller body sizes (Zaret 1980), whether this effect reduces community grazing rate will depend on the concurrent changes in total zooplankton biomass and species composition, as well as algal community size structure.

The presence of fish in our experiment resulted in both lower grazer biomass and a shift to very small cladocerans (primarily *Bosmina*). The average reduction in grazing rates between the fish and no-fish treatments was 19-fold (0.025 versus 0.45/d). This reduction in community grazing rate was due both to the reduction in total cladoceran biomass and the shift to a *Bosmina*-dominated community (which has a lower size-specific grazing rate than either *Ceriodaphnia* or *Daphnia*). The average reduction in cladoceran biomass by fish was 7-fold (0.025 versus 0.175 mg/L), while mass-specific grazing rates were reduced by 2.7-fold (due to *Bosmina's* lower size-specific grazing rate relative to *Daphnia* and *Ceriodaphnia*). Thus, despite a shift to a much less efficient forager, the major effect of fish in reducing zooplankton grazing intensity was due to a reduction in total cladoceran biomass and not to a shift to smaller zooplankton size. We recognize that some error may be associated with our calculated grazing rates, especially for *Bosmina* which have a feeding mode fundamentally different from that of *Daphnia* and *Ceriodaphnia* and show selectivity among small food particles (DeMott 1982; DeMott and Kerfoot 1982). However, given the magnitude of the differences observed, the interpretations should be robust to modest errors in calculated grazing rates.

In our experiment, fish had the same effect on algal standing stock, regardless of whether the initial zooplankton community was composed of large-bodied *Daphnia* or smaller-bodied *Ceriodaphnia*. This was due in part to the fact that the bluegills eliminated both *Daphnia* and *Ceriodaphnia* from the bags. In other systems with either less efficient planktivores, alternate prey, or where the zooplankton can migrate to a hypolimnetic refuge, planktivores may have a differential effect on *Daphnia, Ceriodaphnia*, or other cladocerans. In this case, we would expect the impact of fish to fall most strongly on the large-bodied *Daphnia*, since fish are in general size-selective foragers (see Werner et al. 1983 and Turner and Mittelbach 1990 for examples). The greater vulnerability of large-bodied *Daphnia* to planktivorous fish is no doubt partially responsible for generating the pattern of differential effects on fish on phytoplankton abundance in many studies involving *Daphnia* versus small-cladoceran communities.

Thus, the results of our experiment, and their apparent conflict with previous studies, hinge on two main factors. First, fish were able to eliminate both *Daphnia* and *Ceriodaphnia* from the bags, establishing *Bosmina* as the dominant grazer in both treatments. When this happened, fish significantly enhanced algal standing crop, but the effect was independent of the initial grazer community (i.e. *Daphnia* or *Ceriodaphnia* dominated). Second, the algal community in our bags was composed of only small, highly edible species. In this situation, there was no difference in potential diet breadth between *Daphnia* and *Ceriodaphnia*, and in the absence of fish, both species were able to maintain algae at similar, low levels. This similarity in phytoplankton standing stock occurred despite an average 2.6-fold higher grazing rate for *Ceriodaphnia* relative to *Daphnia*. In fact, the 19-fold difference in grazing rates between the fish and no-fish treatments produced only a 2-fold difference in chlorophyll *a* over the final four sampling dates. These results are consistent with the pattern found in many other studies of grazer manipulations: modest increases in grazing pressure tend to have only small effects on chlorophyll *a* (review in Sterner 1989). For example, in another experiment conducted in the Kellogg Biological Station ponds, we found that a 1.93-fold difference in cladoceran filtering rates between predator and predator-free enclosures produced no detectable difference in algal standing rop between treatments (Turner and Mittelbach 1990). Using the empirical relationship between community filtering rate and chlorophyll *a* determined in the current study (Fig. 4), we can calculate the expected response of chlorophyll *a* to a 1.93-fold change in filtering rate as $1.93^{-0.25} = 0.85$. Thus, a 93% increase in filtering rates would be expected to lead to only a 15% decline in algal standing crop, a difference that would be very hard to detect. The shallow response of algal standing stock to zooplankton grazing is potentially due to a number of factors, the most important of which is the recycling of essential nutrients, especially phosphorus, by the zooplankton (Peters and Rigler 1973; Porter 1976; Lehman 1980; Bergguist and Carpenter 1986; Sterner 1986).

Much effort has been directed towards establishing linkages between components of aquatic food webs and determining the directionality of the interactions (e.g. Kerfoot and Sih 1987; Carpenter 1988). However, in order to predict the outcome of manipulating zooplankton densities or size structure on algal abundance, we must also be able to predict the strength of the interactions among fish, zooplankton, and algae. In this study, we investigated how the magnitude of the algal response to fish manipulations depends on zooplankton species composition. We found that zooplankton species composition had, at most, a weak influence on the fish–algae interaction. In addition, we found that variation in zooplankton filtering rates, independent of species composition, explained most of the variation in algal standing crop. The shallow slope of the relationship between filtering rate and algal abundance indicates that in order to affect algal standing stocks and water clarity, the biomanipulation of zooplankton densities or size structure must cause a large change in overall filtering rates.

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Microbial Leaf Decomposition in Adirondack Streams Exhibiting pH Gradients

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Osgood, M. P., and C. W. Boylen. 1992. Microbial leaf decomposition in Adirondack streams exhibiting pH gradients. Can. J. Fish. Aquat. Sci. 49: 1916–1923.

The effects of water pH and related water chemistry parameters on microbial decomposition processes were studied in three small, low-order woodland streams located in the Adirondack region of New York State. These streams exhibited spatial and seasonal gradients in water pH ranging from pH values of 4.5 to 7.0. Measurements of microbial biomass (ATP), bacterial numbers, and bacterial production (thymidine incorporation) on leaves of *Acer saccharum* were made over a 9-mo period (fall to spring). Decomposition of *A. saccharum* leaves was significantly decreased at lower pH. The developing epiphytic microbial community was affected in a very specific way. Bacterial numbers were not related to water pH values, nor were ATP measurements significantly correlated with pH or any pH-related water chemistry variable. However, thymidine incorporation by bacteria on leaf material (used as a measure of bacterial productivity) was significantly decreased at lower pH. This was the only consistent indicator of reduced microbial activity in response to acidification of these streams.

Nous avons étudié les effets du pH de l'eau et d'autres paramètres chimiques sur les processus de décomposition microbienne dans trois petits cours d'eau d'ordre inférieur, situés dans des zones boisées de la région des Adirondacks, dans l'État de New York. On observait dans ces cours d'eau des gradients spatiaux et saisonniers du pH de l'eau, qui allait de 4,5 à 7,0. Des mesures de la biomasse microbienne (ATP), du nombre de bactéries et de la production bactérienne (incorporation de la thymidine) sur les feuilles d'*Acer saccharum* ont été réalisées sur une période de 9 mo (de l'automne au printemps). La décomposition des feuilles de *A. saccharum* était nettement réduite lorsque le pH était bas. Le développement de la communauté microbienne épiphyte était affecté de façon très particulière. Le nombre de bactéries n'était pas lié au pH de l'eau, et les mesures de l'ATP n'étaient pas corrélées de façon significative au pH ni à une autre variable chimique en rapport avec le pH. Toutefois, l'incorporation de la thymidine par les bactéries dans le matériau végétal constitué par les feuilles (qui sert de mesure de la productivité bactérienne) était réduite de façon lorsque le pH était bas. C'était là le seul indicateur constant d'une réduction de l'activité microbienne en réaction à l'acidification de ces cours d'eau.

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The physical and chemical conditions of small woodland streambeds inhibit the establishment of a significant primary producer community (Petersen and Cummins 1974). Consequently, in many shaded, unpolluted, temperate woodland streams, the energy budget is dominated by the processing of allochthonous organic matter from the surrounding forested watershed. Terrestrial plant leaves contribute as much as 60% of the litter entering a stream (Fisher and Likens 1972). It is generally accepted that leaf decomposition in stream water is largely due to biological action by both the invertebrate and microbial communities, rather than physical factors per se (Webster and Benfield 1986).

In recent years, considerable controversy has developed around the question of whether or not anthropogenic acidification causes any significant impact on forest decomposition processes. Large accumulations of undecayed plant litter were noted as early as 1958 in acid mine-drainage streams in South Africa (Harrison 1958). Lower rates of decomposition and accumulation of plant detritus have been reported in acidified lakes (Francis et al. 1984; Andersson 1985) and streams (Kimmel et al. 1985; Mackay and Kersey 1985; Palumbo et al. 1987b, 1989). In contrast, organic matter decomposition in lake sediments has been reported to be unaffected by acidification of the water column, probably due to the alkalinity generating processes of anaerobic bacteria, which may maintain the lake sediment pH at a more neutral value (Hoeniger 1985; Schindler et al. 1985).

It is unclear whether reduction in organic matter decomposition in acidified waters is due to decreased microbial activity or to reduced abundance of detritus-feeding macroinvertebrates, or both. Numerous studies have reported lower overall numbers and fewer taxa of invertebrates in acidified systems (Andersson 1985; Burton et al. 1985; Simpson et al. 1985). Other studies have reported that microbial numbers or activities associated with decomposing organic matter were reduced at low pH (Traaen 1980; Palumbo et al. 1987b).

The purpose of this study was to clarify the effects of stream acidification on decomposer organisms, especially microorganisms. Measurements of microbial biomass and activity were made in conjunction with measurements of leaf mass loss, macroinvertebrate abundance, and water chemistry parameters.

Materials and Methods

Site Selection and Experimental Design

Five stream sites, representing a gradient in pH and acid-neutralizing capacity (ANC) but comparable in other chemical

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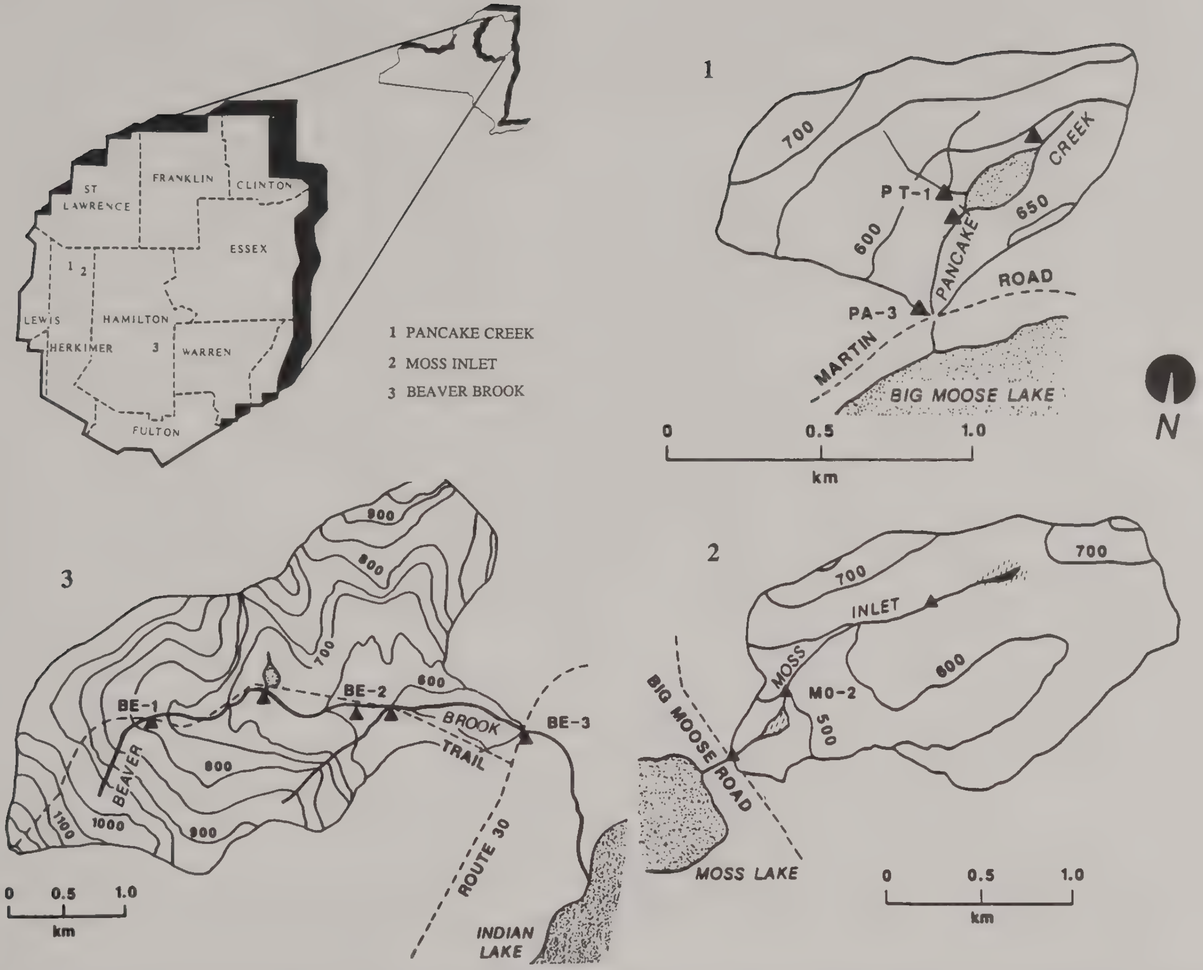
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2 MOSS INLET
3 BEAVER BROOK
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PT-1
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INLET
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FIG. 1. Geographic location of the three study streams. Insert on the top left shows their location in the Adirondack Park of New York State. Map of Pancake Creek on the top right shows sampling site PT1 for the decomposition study. The stippled area in the watershed is a beaver impoundment. Map of Moss Inlet on bottom right shows sampling site MO2. Map of Beaver Brook on bottom left shows sampling sites BE1, BE2, and BE3.

parameters (EPRI 1985), were located in three separate stream basins within the Adirondack Park of New York State (Osgood and Boylen 1990). These sites included three at Beaver Brook (BE1, BE2, and BE3), a tributary site at Pancake Creek (PT1), and a Moss Inlet site (MO2) (Fig. 1).

Standard leaf-pack techniques (Burton et al. 1985) were used to compare detritus decomposition rates among the different streams and sites. Leaf packs were prepared as previously described (Mulholland et al. 1986). Leaves of sugar maple (*Acer saccharum*) (5 ± 0.5 g) were packed in nylon mesh bags (20 × 20 cm, 4-mm mesh) attached to nylon line, 10 bags per line. Five of these lines were placed at each of the stream sites on 21 September 1985 in locations with similar canopy cover and substratum. The lines were tethered to stakes which were then driven into the bottom of each streambed.

Between 1 and 38 wk after initiation of the experiment, one leaf pack was collected from each nylon line from each site. Each pack was collected in a plastic bag submersed beneath each sample, minimizing loss of invertebrates or leaf material when the pack was handled in the water. The leaf packs and water samples were transported on ice to the laboratory at which time the packs were gently rinsed in stream water to remove any silt or invertebrates, which were retained for enumeration and identification. Using a sharpened cork borer, discs (diameter = 1 cm) were cut from the center of the leaf-pack material for microbial analyses. The remainder of the leaf packs were taken to Syracuse University where mass loss measurements and macroinvertebrate counts were done.

Microbiological Procedures

Bacterial numbers were determined using the acridine orange direct count (AODC) technique modified for use on detrital samples (Hobbie et al. 1977). Three samples per site were counted for each sampling date.

ATP was extracted from leaf discs and used as an estimate of total living microbial biomass. Extraction procedures are described in Karl and Craven (1980). Frozen subsamples were later assayed for ATP using Lumac reagents and an integrating photometer (Lumac Biocounter) as described previously (Osgood and Boylen 1990).

Bacterial production on the leaf discs was measured by incorporation of tritiated thymidine into bacterial DNA. The pro-

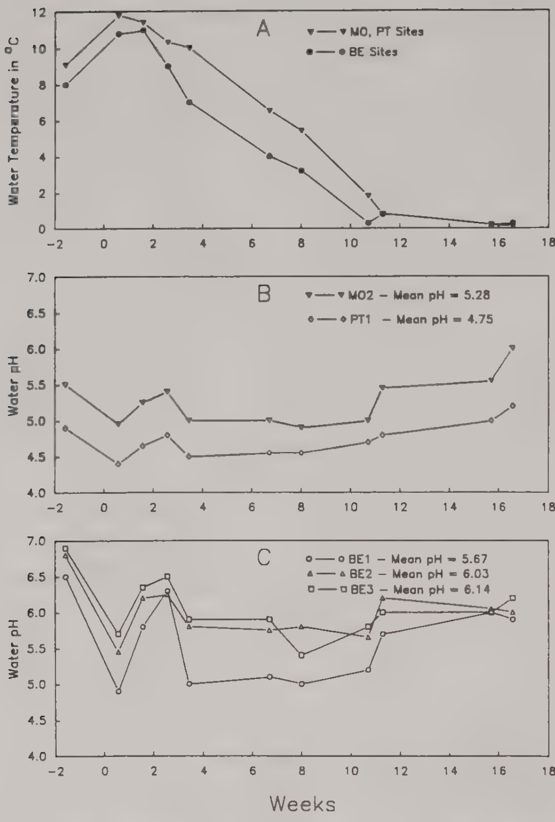

FIG. 2. Water temperature and pH values of the stream sites during the leaf decomposition study. (A) Water temperature values for the decomposition study sites from September 1985 to January 1986; (B) Water pH values for sites MO2 and PT1 from September 1985 to January 1986; (C) Water pH values for sites BE1, BE2, and BE3 from September 1985 to January 1986.

cedure was that of Findlay et al. (1984) as modified by Palumbo et al. (1987b) for use on detrital material. To leaf discs from each pack was added unlabeled thymidine (0.64 nmol) and tritiated thymidine (0.16 nmol, specific activity = 78 × 10^{-5} Ci·nmol^{-1} (1 Ci = 37 GBq), New England Nuclear). Site-specific stream water was added to bring the total liquid volume to 5 mL. The samples were then incubated at stream temperature (±2°C) for 20 min. Overnight extraction of labeled DNA from the bacteria on the leaf material was accomplished by adding 5 mL of a 0.3 N NaOH + 1% sodium dodecyl sulfate (SDS) + 25 mM ethylenediaminetetraacetic acid (EDTA) solution to each sample.

The extracting solution was placed on ice, neutralized to pH 7 – 7.5 with 3 N HC1, and precipitated with 50% trichloroacetic acid (TCA) added to a final concentration of 5% TCA. After centrifugation the precipitate was collected by filtration, hydrolyzed, and radioassayed. Measurements of isotope dilution were done to determine the internal and external pools of thymidine present (Findlay et al. 1984). Only one of the individual estimates of isotope dilution was significantly different from zero. Because of this result, thymidine incorporation was determined at a specific activity that was calculated using 0.80 nmol of thymidine added per sample.

An estimate of total bacterial carbon production over the course of the whole experiment at each site was calculated as follows. The mean of the cell production values (per square centimetre per hour) at the beginning and end of a given time period was multiplied by the number of hours in that period for an interval production estimate. The interval estimates were summed for an estimate of total bacterial carbon produced. Total bacterial carbon production was estimated using a conversion factor of 0.35 pg C·μm^{-3} and a cell volume of 0.222 μm^3 (Palumbo et al. 1987a).

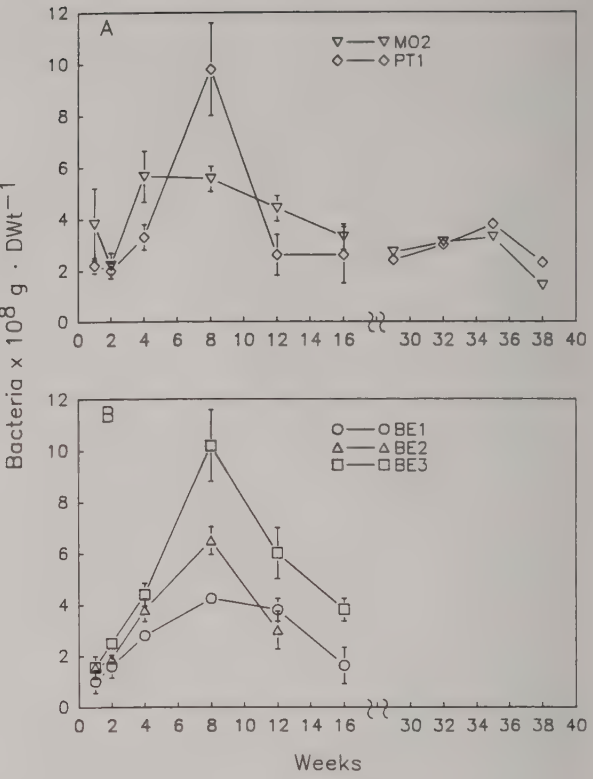

FIG. 3. Bacterial numbers on leaf material. Vertical bars represent 1 SE (n = 3).

Dry weight determinations of leaf discs were performed by standard procedures. Total dry mass measurements were used to calculate the leaf decomposition rate (k) by both linear and exponential decay models as follows (Petersen and Cummins 1974):

$$\text{Dry weight}_t = \text{Dry weight}_0 - k(t)$$

and

$$\text{Dry weight}_t = \text{Dry weight}_0\, e^{-kt}.$$

Analysis of variance (ANOVA) and Tukey pairwise comparison tests were used to determine differences between the microbial measurements at the five sites over the course of the decomposition study. The Pearson product-moment correlation was used to measure the closeness of a linear relationship between two variables.

Results

Temporal Changes in Site Characteristics

As seen in Fig. 2, the water temperature in the three streams dropped from 11–12°C at the end of September to near 0°C in December and January, the steepest decline occurring in

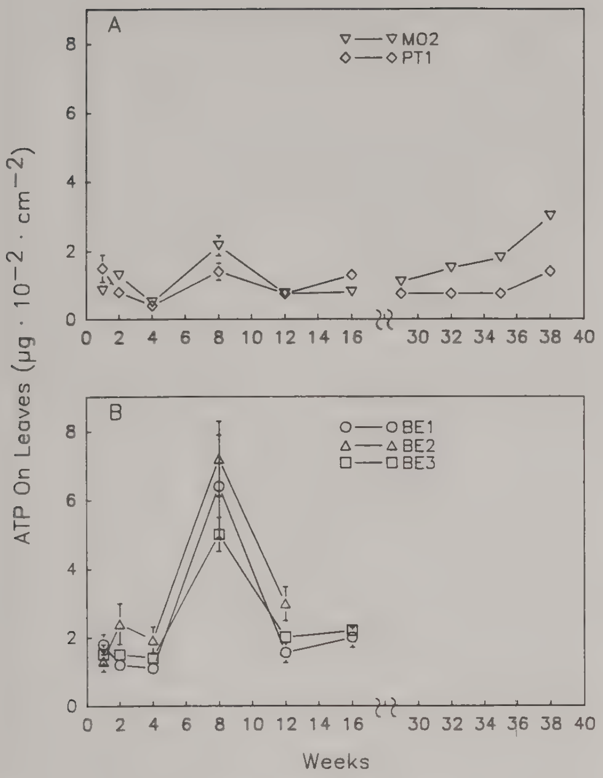

FIG. 4. ATP on leaf material. Vertical bars represent 1 SE ($n = 5$).

October and November. The water pH varied at the sites over the course of the experiment by as much as 1.15 pH units (Fig. 2). At all sites, the lowest pH was measured at week 1, 1 d after a hurricane. The water pH values during the decomposition study were within the range of those seen at these sites for the previous 2 yr, and showed a pattern consistent with previous seasonal pH changes. In general, BE3 had a higher water pH than BE1 and often a higher pH than BE2. All three Beaver Brook sites showed consistently higher water pH values than the Moss Inlet or Pancake Creek site. MO2 water pH values were always higher than those at PT1. The means of the site water pH values over the 16-wk experiment are given in Fig. 2.

Bacterial Numbers on Leaf Material

Bacterial numbers on leaf discs were low for the first 2 wk at all five sites (Fig. 3). Four of the five sites showed peaks on week 8 followed by a decline in numbers to week 16. There were no significant differences in bacterial numbers on leaf discs between sites.

ATP Levels on Leaf Material

Microbial biomass, as measured by levels of ATP per square centimetre of leaf surface area, is shown in Fig. 4. The general pattern for all sites showed low initial levels through week 4 and then peaks of ATP at week 8 at all sites except PT1, which showed no significant changes in ATP throughout the 16 wk of the study. Over the course of the 16-wk study, BE2 had significantly higher ATP levels than BE3, MO2, and PT1, and levels at site BE1 were significantly greater than those at MO2 and PT1.

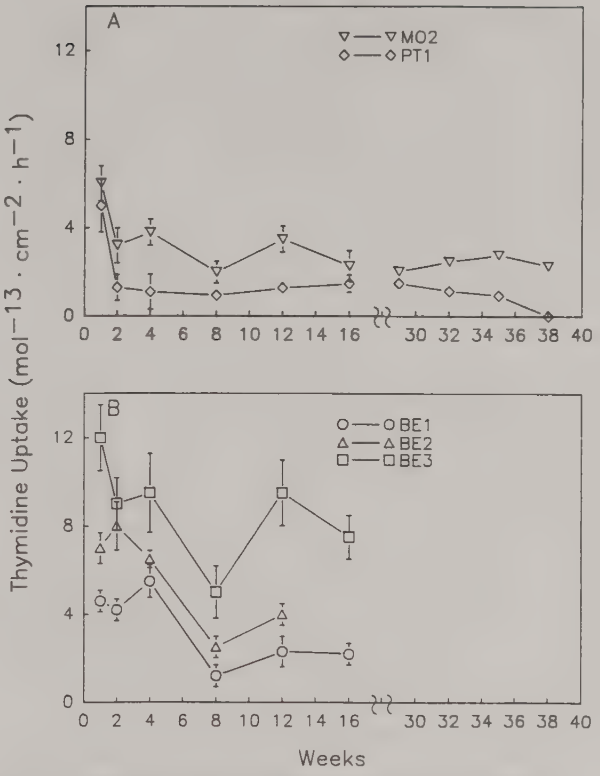

FIG. 5. Tritiated thymidine uptake by bacteria on leaf material. Vertical bars represent 1 SE ($n = 4$).

TABLE 1. Mean bacterial production for the leaf decomposition study. Included are the mean number of bacterial cells produced per day and total bacterial carbon produced over the course of the 16-wk experiment for the five stream sites.

Site	Cells produced ($\times 10^6 \cdot cm^{-2} \cdot d^{-1}$)	Total bacterial carbon produced ($g\ C \cdot g\ dry\ wt^{-1}$)
BE1	33.24	0.102
BE2	43.82	0.133
BE3	73.28	0.218
MO2	28.62	0.085
PT1	13.32	0.040

Bacterial Production on Leaf Material

Uptake of tritiated thymidine by bacteria on leaf discs is reported per square centimetre of leaf surface area (Fig. 5). Thymidine uptake rates were variable between sites, with peak levels of activity occurring after 1 wk of incubation at BE3, MO2, and PT1, after 2 wk at BE2, and after 4 wk at BE1. Site BE3 had significantly greater levels of thymidine uptake than PT1 throughout the experiment, as well as a greater uptake level than other sites on weeks 1, 11, and 16. On week 2, levels of uptake at site BE2 were also significantly higher than PT1, and on week 4, levels at BE1 were higher than at PT1. An overall ANOVA for the entire study showed that thymidine uptake by bacteria at BE3 was significantly higher than at all sites except BE2. PT1 had levels of thymidine uptake that were significantly lower than at all the other sites.

DNA recovery using NaOH, SDS, and EDTA mixture on leaf material, estimated during several of the leaf disc experiments, ranged from 73 to 82%. The thymidine-incorporation data were used to estimate the total bacterial carbon production. Summing the production over the whole 16-wk experiment, the estimates ranged from 0.040 to 0.218 g C·g dry weight leaf material^{-1} (Table 1). At site BE2, there were no leaf packs collected on week 16. For those data, a value for bacterial carbon production was estimated using the mean number of cells produced per hour from all of the previous five sampling dates and multiplying this value by the number of hours between week 11 and 16 for an estimated interval production. This was then added to the other interval estimates.

Decomposition in Spring at Moss Inlet, Site 2, and Pancake Creek Tributary, Site 1

Spring results for the microbiological analyses at these two sites are included in the previous figures. Direct counts of bacteria on leaf material at sites MO2 and PT1 are presented in Fig. 3A. From week 29 to 38, there were no significant differences between the two sites or between sampling dates. The bacterial numbers during this period were similar to those at these two sites during weeks 1, 2, and 16.

ATP levels are presented in Fig. 4A. ATP levels at MO2 were significantly greater than those at PT1 on all three spring sampling dates. On week 38, the ATP measured at MO2 was significantly greater than that on weeks 29 and 35. Both sites had their highest mean level of ATP for the whole study on week 38.

Uptake of tritiated thymidine on the spring sampling dates is shown in Fig. 5A. Overall, uptake was greater at MO2 than at PT1. The levels of tritiated thymidine at MO2 and PT1 were similar to those seen at these sites during the fall–winter study.

Loss of Mass from Leaf Packs

Linear and exponential models fit the mass loss data from week 1 to 16 equally well (r^2 values were similar). The values of the mass loss rate constant, k, are presented as per-day rates (Table 2). A graphical representation of the mass loss of the leaf packs is given in Fig. 6. Over the duration of the study, mass loss measurements of leaf-pack material ranged from 10 to 60%, with constants (k) of $0.00127–0.00709\cdot d^{-1}$ (see Table 2). The mass loss rate constant for site BE3 was significantly greater than that measured at PT1 and BE1. At MO2 and PT1, final k values were 0.00204 and $0.00133\cdot d^{-1}$, respectively, after 38 wk.

During the first week the mass loss constants were much greater than during any other interval. They ranged from $0.00610\cdot d^{-1}$ for MO2 to $0.04319\cdot d^{-1}$ for BE2, and the mean for the five sites was $0.01966\cdot d^{-1}$ (see Table 2). These numbers translate into percent mass loss values of 4–26% with a mean of 12.4%. Over the remainder of the 16-wk experiment, the further mass loss from week 1 to 16 was 9% at BE1, 21% at BE3, 6% at MO2, and 5% at PT1. From week 1 to 11, the percent mass loss at BE2 was 46%. These percent loss values are based on the mass of the leaf material remaining after week 1, not on the initial mass of the leaf packs. The dry mass of the leaf packs on the last sampling date is also presented in Table 2. Although the reported weight for BE2 is from week 11, it is significantly less than for all other sites. BE3 is significantly less than PT1 and MO2 (week 16), but not BE1.

Correlations of Water Chemistry Parameters with Microbiological Parameters

Pearson correlation coefficients between the water chemistry and microbiological variables from the individual sites were calculated and are shown in Table 3. The uptake of tritiated thymidine was positively correlated ($p < 0.0001$) with pH, ANC, Ca, and Mg and negatively correlated ($p < 0.0001$) with total, monomeric, and organic Al. Thymidine uptake was less highly correlated with Si and Na and was negatively correlated with dissolved organic carbon (DOC) and total fluoride. Levels of ATP and numbers of bacteria on the leaf material were all significantly correlated with several water chemistry variables. The biological variables were not related to each other. Numbers of bacteria were significantly and negatively correlated ($p < 0.05$) with the mass loss rate, k. ATP levels were negatively correlated ($p < 0.05$) with the leaf-pack weight.

Discussion

Although the study sites were chosen to be as uniform as possible, the restrictions imposed by the low-water conditions present in September did not allow for complete homogeneity. The three sites at Beaver Brook were actually considered to be riffle areas whereas the sites at Moss Inlet and Pancake Creek were pools, with deeper and quieter water. This difference

TABLE 2. Calculated dry weight loss constants, k. Standard errors of k and r^2 values are based on the exponential decay model for leaf mass loss between 7 and 112 d in each stream. Also presented are the week 1 k values, mean dry weights of leaf material on the last sample day, the standard error, and the total percent loss dry weight on that day. The initial mean weight of the leaf packs was 5.02 g.

	k (d^{-1})				Dry wt on last day (g)		
Site	Mean	SE	r^2	Week 1 k value	Mean	SE	Loss (%)
BE1	0.00127	0.00109	0.586	0.01626	4.09	0.13	19
BE2[a]	0.00709	0.00090	0.976	0.04319	1.99	0.40	60
BE3	0.00275	0.00068	0.720	0.02212	3.39	0.19	32
MO2	0.00326	0.00178	0.199	0.00610	4.53	0.09	10
PT1	0.00160	0.00063	0.340	0.01063	4.45	0.22	11
MO2[b]	0.00240	0.00114	0.881		3.71	0.26	26
PT1[b]	0.00133	0.00051	0.857		3.61	0.23	28

[a] Values for BE2 are for leaf mass loss between 7 and 77 d. No packs were collected at this site after this.

[b] These values for sites MO2 and PT1 are calculated using all the data for these sites through the June 1986 sampling.

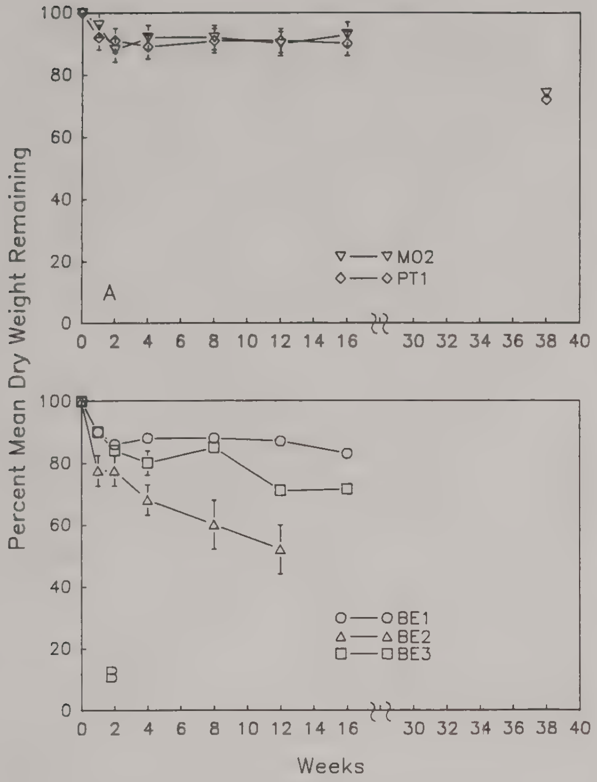

FIG. 6. Mass loss of leaf packs. Vertical bars represent 1 SE ($n = 5$).

between sites may have had an effect on the decomposition of the leaf packs by introducing an unmeasured difference in mechanical action between sites.

The numbers of bacteria directly counted on the leaf material were low compared with those reported in other studies that used similar counting methods (e.g. Palumbo et al. 1987b, 1989). In the present study, we saw a peak of bacterial numbers at weeks 4–7. Francis et al. (1984), in a study of Adirondack lakes lasting 2 yr, described a pattern of increase in bacterial numbers on leaves up to 35 wk and then a stabilization of numbers. Palumbo et al. (1987b, 1989) found a pattern of increasing numbers over the first 4 wk of their 15-wk study of streams in eastern Tennessee followed by a relative plateau. They observed a peak of directly counted bacteria at 10 wk.

Other studies of microbial colonization of leaf detritus in aquatic systems have indicated that fungi are the initial colonizers and that bacterial numbers are low initially and then increase steadily (Benner et al. 1986). The results presented here also show slow initial bacterial colonization. However, in our present study, we did not enumerate fungal colonization. A possible explanation for the decrease in bacterial numbers seen at all sites after the peaks at week 4 or 8 is the exhaustion of labile carbon in the leaf pack.

Bacterial numbers were not reduced by low pH in the present study. The two highest mean values were seen at BE3, the highest pH site, and at PT1, the lowest pH site. The lowest mean number of bacteria was also counted at BE3. These results are consistent with those of Palumbo et al. (1987b, 1989) who found no significant differences between bacterial numbers on leaves at four stream sites with differing pH. Francis et al. (1984), however, found that leaf litter from a low-pH lake frequently had fewer bacteria than that from lakes with higher pH values.

Enumeration of bacteria cannot serve as a reliable measure of their relative importance in decomposition processes. For example, we found no relationships between numbers of bacteria and thymidine uptake, a measure of bacterial activity. The constantly changing nature of bacterial populations, in terms of species and levels of activity, makes estimates of population size of limited use in assessing the dynamic functioning of the communities.

Microbial biomass on leaves as measured by ATP levels has not been found to be particularly sensitive to water pH or related chemical parameters (Rosset and Barlocher 1981; McKinley and Vestal 1982). ATP on leaves was not a sensitive indicator of microbial community response to short-term changes in water pH (Palumbo et al. 1987b, 1989) although highest periphyton ATP levels occurred at the site with the highest pH (6.5, compared with 5.8, 5.0, and 4.6; Mulholland et al. 1986). There were no significant differences in ATP levels among these lower pH sites. ATP measurements obtained in our study of Adirondack streams did not vary consistently with pH and were not significantly correlated with pH or any pH-related chemical variable.

Physical factors have been found to affect ATP levels on leaves. Increases in temperature and turbulence were both found to significantly increase ATP on *Carex* litter in microcosm studies (Federle et al. 1982). In the present study, temperature was not significantly correlated with ATP levels. However, given the difference in incubation conditions in the Beaver Brook riffle and Pancake Creek and Moss Inlet pools, it is possible that the differences in water current, while not quantified, influenced ATP levels. Mechanisms proposed for such an effect include an increased rate of exchange of nutrients, dissolved oxygen, and waste materials with increased turbulence or an increase in the disintegration of detrital material due to increased water turbulence providing more surface area for microbial colonization (Federle et al. 1982). Examination of the leaf material in the packs over the course of our study did show more rapid comminution at the Beaver Brook sites which also generally had higher levels of ATP on the leaf material than the Moss or Pancake sites.

Thymidine incorporation on leaf material and the inferred bacterial production appear to be significantly related to water pH and pH-related chemistry variables in the present study. The thymidine uptake at the highest pH site, BE3, was higher than at all the other sites on almost all dates, and the differences were statistically significant during much of the experiment. The most acidic site, PT1, had lower levels of thymidine uptake than all other sites throughout the experiment, and again, these differences were frequently significant. The other three sites, which had more similar mean water pH values, had similar thymidine uptake levels. In agreement with these results, correlation coefficients between thymidine uptake and pH and pH-related chemistry variables were highly significant.

The incorporation of tritiated thymidine into DNA has been used for bacterial studies on decomposing leaf litter in two studies in Tennessee (Palumbo et al. 1987b, 1989). Results from these investigations indicated reduced bacterial production due to acid conditions. Reduced thymidine uptake was the only parameter measured that consistently varied with water pH in our Adirondack study. These results are in agreement with those of other studies in which bacterial activity was found to be more sensitive to acidification than numbers or biomass (Carpenter et al. 1983; Benner et al. 1985). Productivity measurements

TABLE 3. Matrix of Pearson correlation coefficients between the chemical and microbiological variables. All chemical variables are identified as follows. Biological and physical variables: AVEATP = ATP, AVETDR = thymidine uptake, AVEOX = oxygen consumption. AVEAOS = directly counted bacteria, KVAL = mass loss rate, *k*, MASS = leaf-pack weight. TEMP = water temperature, ANC = acid-neutralizing capacity, DIC = dissolved inorganic carbon, DOC = dissolved organic carbon, TOTF = total fluoride, TOTAL = total aluminum, MONOAL = monomeric aluminum, TOTP = total phosphorus, SRP = soluble reactive phosphorus, COND = conductivity, ORGAL = organic aluminum. All other chemistry variables are equal to the total concentration of the metal or ion. †$p < 0.05$; ††$p < 0.01$; *$p < 0.001$; **$p < 0.0001$.

Variable	TEMP	pH	ANC	DIC	DOC	Na	K	Ca	Mg
AVEATP	−0.16140	0.06678	0.03582	−0.31701	−0.13911	−0.33817†	−0.40028†	−0.15986	−0.08486
AVETDR	0.10265	0.76734**	0.80262**	0.34770†	−0.50678††	0.41176†	−0.12383	0.75766**	0.80541**
AVEOX	0.20360	−0.06622	−0.17620	−0.29326	0.18411	−0.45171††	0.14647	−0.25238	−0.23961
AVEAOS	−0.26737	−0.25036	−0.25919	−0.35493†	0.18112	−0.50999††	−0.12891	−0.20746	−0.25663
KVAL	0.41108†	0.49229††	0.59698*	0.38497†	−0.20120	0.35235	−0.04996	0.41771†	0.48930††
MASS	0.28189	−0.44161†	−0.36705	−0.02314	0.49414††	−0.17887	0.27119	−0.20210	−0.21785

Variable	TOTF	Cl	NO_3	SO_4	NH_4	Si	TOTAL	MONDAL	TOTP
AVEATP	−0.10320	−0.12059	−0.15003	−0.34797†	−0.10346	−0.22348	−0.23555	−0.17940	−0.19035
AVETDR	−0.35249†	0.15710	−0.32146	−0.05015	−0.19366	0.55004*	−0.65613**	−0.64537**	−0.10149
AVEOX	−0.06444	−0.14499	−0.33124	0.12094	−0.06368	−0.26421	0.09281	0.10592	−0.09494
AVEAOS	0.19878	0.43442††	−0.01720	0.15310	−0.00479	−0.32521	0.19630	0.22378	−0.10616
KVAL	−0.19514	−0.23656	−0.08391	−0.25762	−0.14372	0.63704*	−0.31567	−0.33095	−0.03807
MASS	0.44584†	0.21378	−0.02667	0.50732††	0.18018	−0.22098	0.45697†	0.45998†	0.09579

Variable	SRP	COND	ORGAL	AVEATP	AVETDR	AVEOX	AVEA0S	KVAL	MASS
AVEATP	0.08866	−0.40017†	0.34986†	1.00000					
AVETDR	−0.30983	−0.14218	−0.61449**	−0.11250	1.00000				
AVEOX	0.11160	−0.22345	0.23338	0.16394	−0.14246	1.00000			
AVEAOS	−0.23095	0.01316	0.58973*	0.27864	−0.16068	0.23056	1.00000		
KVAL	0.01833	0.16439	−0.33282	−0.03497	0.31988	−0.09288	−0.37256†	1.00000	
MASS	0.24043	0.37064†	−0.17203	−0.37004†	−0.24524	−0.16509	−0.05503	−0.18266	1.00000

provide a more complete estimate of total bacterial activity. Unlike assays for heterotrophic uptake, which measure only the activity of the portion of the population that is able to use the substrate provided, thymidine is taken up by a large number of aerobic bacterial species. Our results indicate that lowered pH affects a large portion of the bacterial community.

The leaf-pack mass loss rates in this study were "slow" as defined by Petersen and Cummins (1974) except for BE2, which had a "medium" rate. Water temperatures were not significantly correlated with mass loss rates in this study. The leaf-pack losses we observed did seem to be related to pH. Both the mass loss rates and the leaf-pack weights were significantly correlated with pH and several pH-related chemistry variables (see Table 3). Comparisons of the amount of leaf litter remaining on the last sampling date (of the 16-wk study) can give a good indication of differences in decomposition between sites (Carpenter et al. 1983). Using this criterion, site BE3, the highest pH site, showed a significantly greater level of decomposition than MO2 and PT1, the lowest pH sites, but this level was not significantly different from BE1. BE2 showed a significantly higher decomposition level than all other sites. These results are in agreement with those of other researchers who found that leaf decomposition rates decreased with decreasing pH (McKinley and Vestal 1982; Burton et al. 1985; Kimmel et al. 1985; Mulholland et al. 1986).

Decomposition of organic matter is dependent on both microorganisms and macroinvertebrates, and reduced pH can perturb both communities. The macroinvertebrate data collected by researchers at Syracuse University in conjunction with this study did not indicate a reduction in macroinvertebrate numbers at low pH. Densities of shredders were low at all sites (M. Smith, pers. comm.). Other researchers have demonstrated the importance of long-term experiments (greater than 80 d) in documenting effects of acidification on invertebrates (Burton et al. 1985). Even our Adirondack study might not have been of sufficient length (>112 d) to determine macroinvertebrate sensitivity to acidification in terms of growth and recruitment.

In conclusion, the decomposition of *A. saccharum* leaves, as indicated by the leaf mass loss results, was significantly depressed in stream sites with low water pH values. The decomposer community most affected was microbial, not invertebrate, in nature. Specifically, bacterial production was reduced by acidic conditions. Such inhibition of detrital processing will have consequences throughout the stream ecosystem, interfering in nutrient recycling processes and in primary and detritovore production.

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Temporal and Spatial Distribution of the Harmful Diatoms *Chaetoceros concavicornis* and *Chaetoceros convolutus* along the British Columbia Coast

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Albright, L. J., S. Johnson, and A. Yousif. 1992. Temporal and spatial distribution of the harmful diatoms *Chaetoceros concavicornis* and *Chaetoceros convolutus* along the British Columbia coast. Can. J. Fish. Aquat. Sci. 49: 1924–1931.

The harmful phytoplankters *Chaetoceros concavicornis* and *Chaetoceros convolutus* are normal components of the phytoplankton assemblage along the British Columbia coast. During 1988, these diatoms occurred at their highest concentrations in the spring and fall from the Strait of Georgia to Queen Charlotte Strait. North of Queen Charlotte Strait, the maximal concentrations appeared to be delayed until summer. The greatest concentrations occurred in the waters of the Strait of Georgia and its contiguous bays and inlets, especially Jervis Inlet, where concentrations of *C. concavicornis* and *C. convolutus* which could be harmful to salmon occurred during the spring and fall of 1988. Lesser concentrations occurred in coastal seawaters north of the Strait of Georgia. These phytoplankters were absent, or were present in only minor concentrations, in inlets with low-salinity waters such as Holberg Inlet and Indian Arm. Harmful *Chaetoceros* cells were restricted to seawaters greater than approximately 17‰ salinity. Vertically and horizontally well-mixed waters, such as those of Johnstone Strait, also appeared to restrict the presence of these cells. The greatest concentrations of these diatoms occurred during the spring and fall in relatively stratified coastal seawater with salinities between 26 and 34‰.

Les phytoplanctontes dangereux que sont *Chaetoceros concavicornis* et *Chaetoceros convolutus* sont des éléments normalement présents dans l'assemblage phytoplanctonique le long de la côte de la Colombie-Britannique. En 1988, ces diatomées ont atteint leurs concentrations les plus élevées au printemps et à l'automne du détroit de Géorgie au détroit de la Reine-Charlotte. Au nord du détroit de la Reine-Charlotte, les concentrations maximales semblent avoir été retardées jusqu'à l'été. Les concentrations les plus élevées ont été observées dans les eaux du détroit de Géorgie et dans les baies et inlets adjacents, particulièrement l'inlet Jervis, où on a mesuré au printemps et à l'automne 1988 des concentrations de *C. concavicornis* et *C. convolutus* qui pouvaient être dangereuses pour le saumon. Des concentrations plus faibles ont été observées dans les eaux côtières au nord du détroit de Géorgie. Ces phytoplanctontes étaient absents, ou présents en faibles concentrations, dans les inlets à faible salinité comme l'inlet Holberg et le bras Indian. Les cellules dangereuses de *Chaetoceros* étaient confinées aux eaux marines présentant une salinité supérieure à environ 17 ‰. Les eaux bien mélangées dans les plans vertical et horizontal, comme celles du détroit de Johnstone, semblaient aussi limiter la présence de ces organismes. Les concentrations les plus élevées de ces diatomées ont été observées au printemps et en automne dans des eaux côtières relativement stratifiées présentant des salinités situées entre 26 et 34 ‰.

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More than 50 species of the diatom genus *Chaetoceros* have been found in seawater off the west coast of North America (Cupp 1943; Gaines and Taylor 1986). All of these *Chaetoceros* spp. possess spines, but it is those species which have spinules (barbs) on their spines that are harmful to finfish, including wild and penned salmon (Gaines and Taylor 1986). The *Chaetoceros* sp. which has been reported to most commonly cause mortalities of penned salmon in British Columbia coastal waters is *Chaetoceros convolutus* (Bell et al. 1974; Kennedy et al. 1977; Brett et al. 1978), although F. J. R. Taylor (Department of Oceanography, University of British Columbia, Vancouver, B.C., pers. comm.) reports that *Chaetoceros concavicornis* (which also possesses spines with spinules) kills penned salmon as well.

In the case of lingcod (*Ophiodon elongatus*), the ''barbed'' spines of these diatoms appear to become imbedded in the secondary lamellar cells of the gills (Bell 1961). There they are held by the spinules. The imbedded spines cause production of excessive mucus and hyperplasia and hypertrophy of the secondary lamellar cells. The impairment of these cells may indirectly cause death of salmon by limiting gill function (C. Yang and L. J. Albright, pers. obs.) Bell et al. (1974) found that lethal damage to penned salmon appeared to occur at *C. convolutus* concentrations of approximately 5 cells·mL^{-1} and greater.

Since 1984, there has been a rapid development of salmon farms along the southern coast of British Columbia, especially on the west coast of Vancouver Island, the Strait of Georgia, and Johnstone and Queen Charlotte straits (Fig. 1). Reports of penned salmon killed by harmful *Chaeteoceros* spp. quickly followed the establishment of many of these farms. Anecdotal information within the salmon farming community indicates that several areas which are currently being farmed on the southern British Columbia coast are more prone to recurrent blooms of harmful *Chaetoceros* spp. than are others.

Knowledge of harmful *Chaetoceros* spp. concentrations within coastal British Columbia seawaters would allow one to (1) better control penned salmon losses due to these phyto-

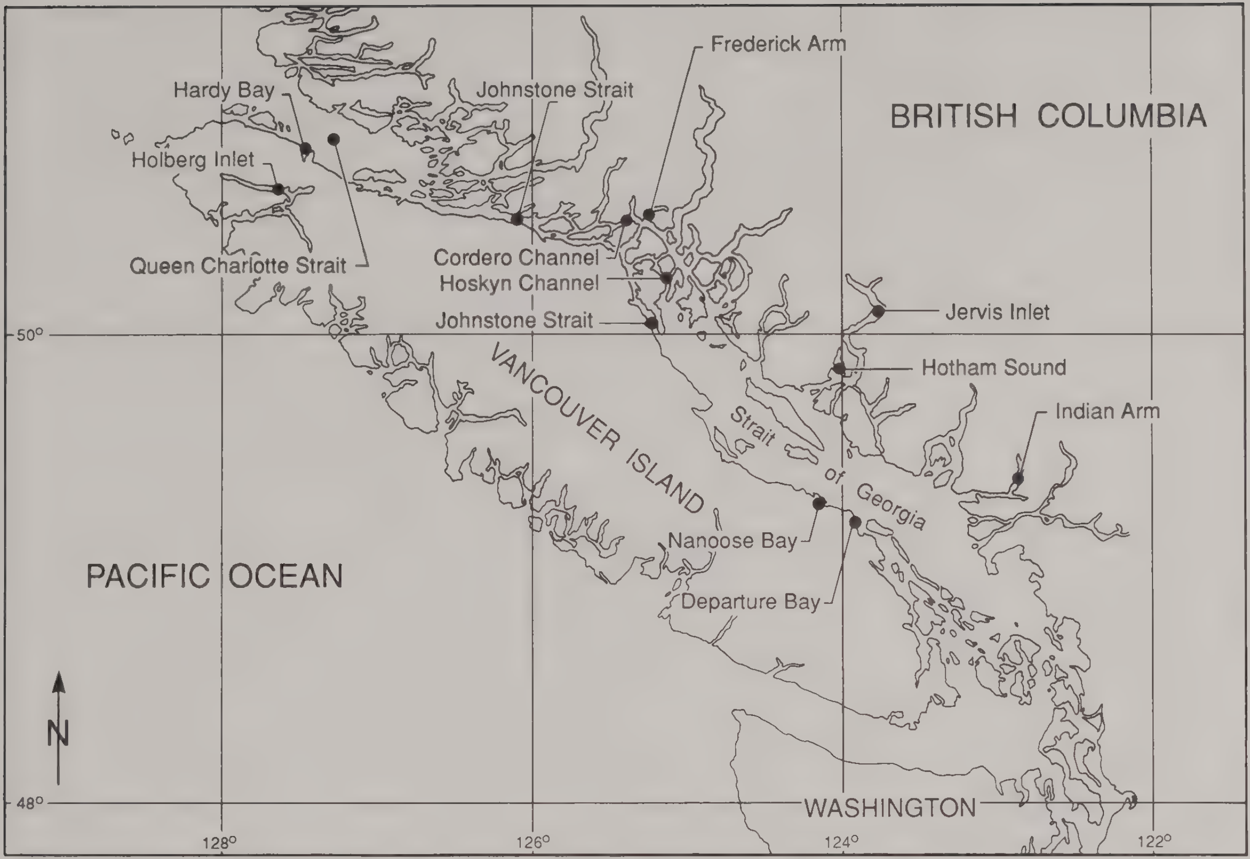

FIG. 1. Chart of the Strait of Georgia and Johnstone and Queen Charlotte straits showing the sampled farm sites.

plankters and (2) avoid establishing a farm in waters prone to these harmful phytoplankton blooms. This study was therefore initiated to (1) determine the temporal and spatial concentrations of *C. concavicornis* and *C. convolutus* in the surface seawaters of coastal British Columbia with particular emphasis on the Strait of Georgia and Johnstone and Queen Charlotte straits and their contiguous inlets and (2) identify some of the factors which may regulate the concentrations of harmful *Chaetoceros* spp. in various British Columbia coastal surface waters.

Materials and Methods

Temporal Survey

Sites selected within Indian Arm, Hotham Sound, Hoskyl Channel, Cordero Channel (near Horn Point), Frederick Arm, Hardy Bay, and Holberg Inlet (Fig. 1) were routinely sampled (at least once per week) for a minimum of several months to determine temporal changes in the concentration of harmful *Chaetoceros* spp. (i.e. *C. concavicornis* and *C. convolutus*). For each sample, a 10-m vertical water column was sampled using a 20-μm-mesh Wisconsin net. Samples were preserved using Lugol's solution, returned to the laboratory, and subsampled. In total, three 10-μL subsamples were examined at 48× total magnification and the number of harmful *Chaetoceros* cells counted using an inverted light microscope and the Utermöhl (1958) technique (25 mL of preserved sample was settled within the Utermöhl chamber for 24 h prior to counting). Concentrations within the 0- to 10-m water column were calculated based upon the volume of water sampled, the volume of sample in the cod end, and the volume of the subsample. No attempt was made to discriminate between *C. concavicornis* and *C. convolutus*.

Areal Survey

Seventy-four stations (numbered 1–74) were selected within the Strait of Georgia and Johnstone and Queen Charlotte straits and their contiguous inlets (Fig. 2), such that a representative number of samples were retrieved from each water body. Water samples were collected from these stations during the periods of 11–18 March, 2–8 April, and 4–12 October 1988. An additional set of stations was selected in the northern portion of Queen Charlotte Strait to Chatham Sound (latitude approximately 54°N) on the central and northern coasts of British Columbia (Fig. 3). Water samples were collected at these stations during the periods of 10–22 August 1988 and 20–24 April 1989. At each station, a 10-m vertical water column was sampled using a 1.25-cm internal diameter hose. Temperature and salinity of each sample were determined using a thermometer and a hand-held refractometer (American Optical), respectively. Samples were then preserved in Lugol's solution and returned to the laboratory where counts of harmful *Chaetoceros* cells were made using the Utermöhl (1958) technique.

Culture of *C. convolutus*

Culture media with salinities of 5, 10, 15, 20, 25, 30, 35, and 40‰ were made by dissolving inorganic salts (in the pro-

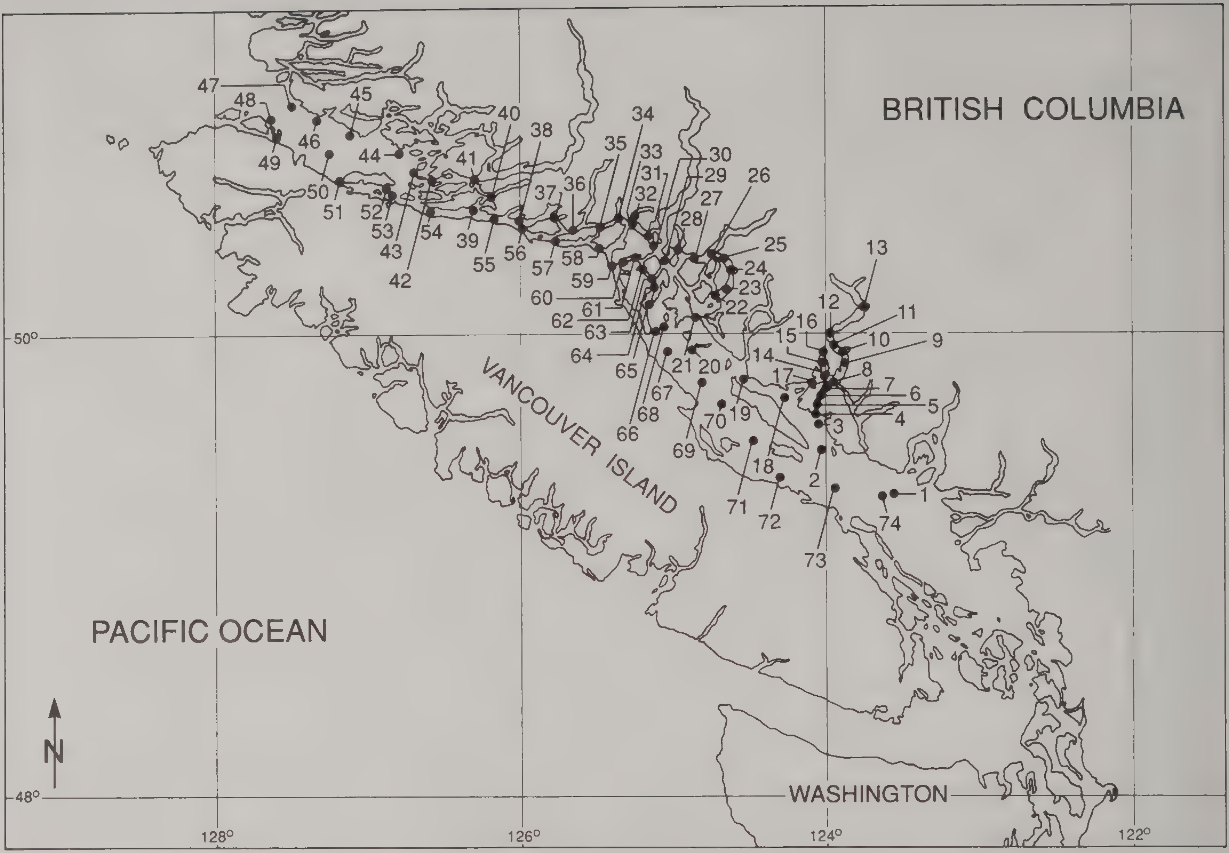

FIG. 2. Chart of the Strait of Georgia and Johnstone and Queen Charlotte straits showing the 11–18 March, 2–8 April, and 4–12 October 1988 sampling stations.

portions given by Harrison et al. 1980) in 100 mL of distilled water, each contained in a 250-mL Erlenmeyer flask. Each medium was then enriched with an equal amount of vitamins, minerals, and trace metals in the concentrations specified in Harrison et al. (1980). The pH of each solution was adjusted to approximately 8.0. Salinities were checked using a hand-held refractometer.

Each flask (three per salinity) was inoculated with 10 mL of a unialgal culture of *C. convolutus* (No. 619, Northeast Pacific Culture Collection, University of British Columbia) which had been cultured in enriched natural seawater medium (Harrison et al. 1980) at a salinity of 30.5‰ for 7 d. After addition of the inoculum, final culture salinities were 8, 12, 17, 22, 27, 32, 36, and 40‰. Cultures were incubated at 10–13°C under constant illumination with an irradiance of $28.5\ \mu\mathrm{Ein\cdot m^{-2}\cdot d^{-1}}$. A 3-mL sample was removed from each flask every 3–4 d and the cells stained with 3 mL of a 0.1% (w/v) acridine orange solution for 15 min. Each stained sample was then filtered through a 25-mm-diameter, 2.0-μm pore size Nuclepore filter. Filters were mounted in low-fluorescence immersion oil on a glass slide and examined at 100× magnification using a Zeiss Axioplan epifluorescent microscope. All intact cells within each of ten 1-mm^2 grids (selected at random) were counted and the concentration of cells in each flask calculated from the mean cell count of these grids.

Results

Temporal Survey

Strait of Georgia to Queen Charlotte Strait

In the spring of 1988, harmful *Chaetoceros* cells were first reported to occur in March.

Hotham Sound, a water body contiguous to Jervis Inlet (Fig. 1), had three peaks in the abundance of harmful *Chaetoceros* cells during the sampling period of 1 March to 6 June 1988 (Fig. 4). During the latter half of April, *Chaetoceros* cells were most abundant, with the concentration of cells exceeding the level ($>5\ \mathrm{cells\cdot mL^{-1}}$) considered to be overtly harmful to salmonids. No harmful *Chaetoceros* cells were reported from 6 June through to the end of the sampling in August.

During 1988, harmful *Chaetoceros* cells in Hoskyn Channel, a water body between Quadra and Read islands in the southern Strait of Georgia (Fig. 1), were first reported in March and occurred relatively frequently in samples up to the termination of sampling in August (Fig. 4). With the exception of a peak in abundance ($5.6\ \mathrm{cells\cdot mL^{-1}}$) in early June, harmful *Chaetoceros* cells were present in lower numbers than in Hotham Sound. In 1988, the abundance of harmful *Chaetoceros* cells did not exceed the level considered to be overtly harmful to salmonids. However, on several occasions in April and May

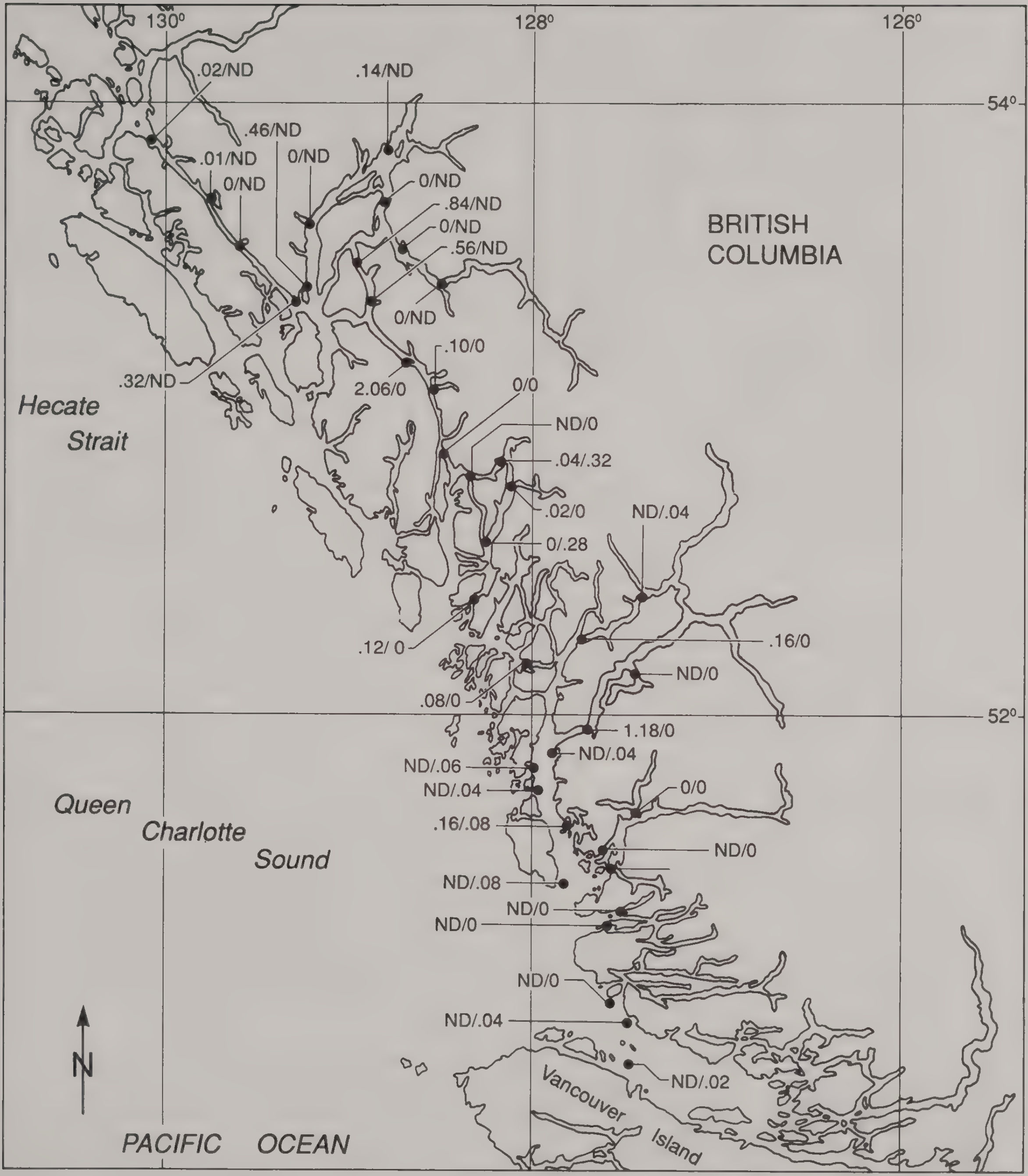
130°
128°
126°
54°
52°
.02/ND
.14/ND
.46/ND
.01/ND
0/ND
0/ND
0/ND
.84/ND
0/ND
.56/ND
0/ND
BRITISH
COLUMBIA
.10/0
2.06/0
.32/ND
Hecate
Strait
0/0
ND/0
.04/.32
.02/0
ND/.04
0/.28
.12/ 0
.16/0
ND/0
.08/0
1.18/0
ND/.04
ND/.06
ND/.04
0/0
.16/.08
Queen
Charlotte
Sound
ND/0
ND/.08
ND/0
ND/0
ND/0
ND/.04
ND/.02
N
Vancouver
Island
PACIFIC OCEAN

FIG. 3. Mean concentrations (cells·mL^{-1}) of harmful *Chaetoceros* spp. within the 0- to 10-m water column at various locations along the central and northern coasts of British Columbia during 10–22 August 1988 (left of slash) and 20–24 April 1989 (right of slash).

1987, the concentrations of harmful *Chaetoceros* cells exceeded the level considered to be harmful to salmonids.

In Hardy Bay, a water body contiguous to Queen Charlotte Strait (Fig. 1), harmful *Chaetoceros* cells were reported in low abundance throughout the early summer and fall (Fig. 4). During this time, harmful *Chaetoceros* abundance never exceeded 1.3 cells·mL^{-1}.

The water of Cordero Channel at Horn Point had a very minor spike in harmful *Chaetoceros* concentrations for several days in mid-August 1988 (unpublished data) whereas the water of Frederick Arm (a bay contiguous to Cordero Channel and approximately 3 km from Horn Point) had minor peaks in harmful *Chaetoceros* spp. during May through July 1988 (Fig. 4).

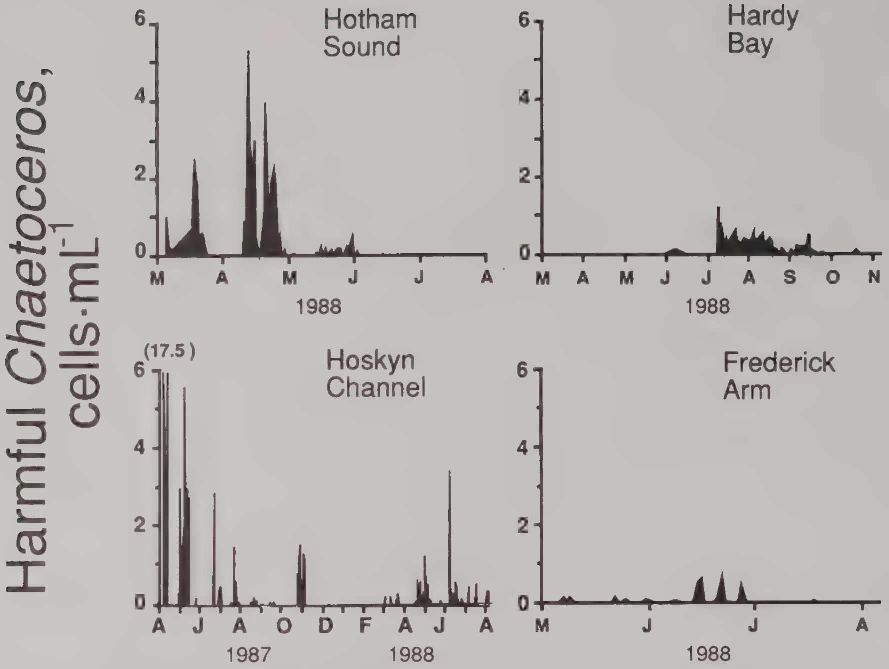

FIG. 4. Harmful *Chaetoceros* concentrations at Hotham Sound (station 16, Fig. 2) for the period of 1 March to 6 June 1988, Hoskyn Channel (station 63, Fig. 2) for the period of 5 April 1987 to 1 August 1988, Hardy Bay for the period of 1 March to 31 October 1988, and Frederick Arm (station 33, Fig. 2) for the period of 1 May to 10 August 1988. Samples were taken at least once per week and represent cell numbers for the 0- to 10-m vertical water column sampled with a 20-μm-mesh net.

Harmful *Chaetoceros* spp. were not observed within the water of Indian Arm except during the periods of 25–31 March and 2–7 May 1989. The maximum concentration was 0.020 cell·mL^{-1}.

Harmful *Chaetoceros* cells were not observed in water samples retrieved from Holberg Inlet during the period of 1 April to 31 December 1988.

Generally, the greatest concentrations of harmful *Chaetoceros* cells along the southern British Columbia coast were found in surface seawaters sampled during the October 1988 cruise (Fig. 5).

Queen Charlotte Strait to Chatham Sound

Harmful *Chaetoceros* cells were observed in waters retrieved from many locations along the central and northern coasts of British Columbia (Fig. 3). The mean concentrations of these phytoplankters from Queen Charlotte Strait to Chatham Sound were greater in midsummer 1988 than in spring 1989.

Areal Survey

Strait of Georgia to Queen Charlotte Strait

The concentrations of harmful *Chaetoceros* cells within the 0- to 10-m water column along the southern coast of British Columbia (stations 1–74, Fig. 2) during the March, April, and October 1988 sample periods were greatest in the waters of Jervis Inlet, with lesser concentrations occurring in the waters of the Strait of Georgia (Fig. 5). Considerably lower concentrations were found in the waters of Queen Charlotte and Johnstone straits, although harmful concentrations of *Chaetoceros* were found in water removed from the vicinity of Call Inlet (stations 39 and 40, Fig. 2) during the October cruise (Fig. 5).

Queen Charlotte Strait to Chatham Sound

Harmful *Chaetoceros* spp. were observed to occur in various water bodies along the central and northern British Columbia coasts, although the concentrations noted were generally considerably less than those sampled along the southern coast (Fig. 3, cf. Fig. 2 and 5). No single water body could be identified as having greater concentrations than another along the central and northern coasts, as was seen in water sampled from the southern coast (see above). The spring 1989 concentrations of these harmful *Chaetoceros* spp. were lower than the summer 1988 concentrations.

Effect of Salinity on the Presence of Harmful *Chaetoceros* in Natural Seawater

When the salinities of the water samples from British Columbia coastal waters during the five cruises (Fig. 2 and 3) were plotted against the concentration of harmful *Chaetoceros* cells present (Fig. 6), these phytoplankters were not observed in seawaters of salinity 17‰ and less. Between salinities 17 and 22‰, these cells were present in many waters, but at low concentrations. Maximum concentrations were observed in waters with salinities between 25 and 34‰ (Fig. 6).

In vitro Growth of *C. convolutus* at Various Salinities

Under laboratory conditions, unialgal cultures of *C. convolutus* grew well between 22 and 36‰ salinity but not at or below 17‰ salinity when incubated at a temperature of 10–13°C. At 40‰ salinity, limited growth was observed. The optimum salinity for growth was approximately 27‰ (Fig. 7).

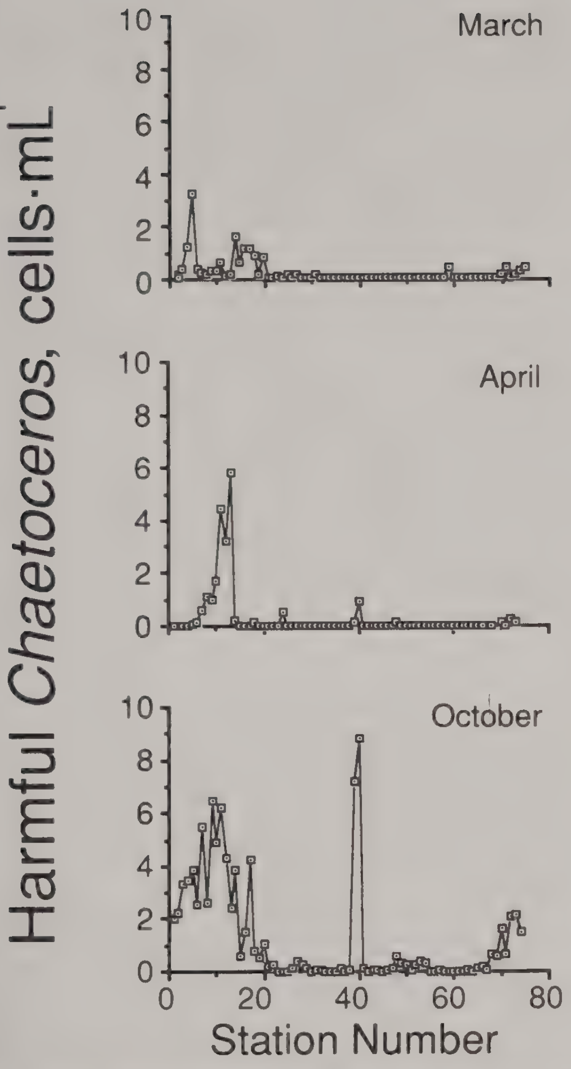

FIG. 5. Mean concentrations of harmful *Chaetoceros* spp. within the 0- to 10-m water column at stations 1–74 (see Fig. 2 for station locations) along the southern coast of British Columbia during the March, April, and October 1988 sample periods.

Discussion

The waters of Jervis Inlet and the northern portion of the Strait of Georgia and their contiguous inlets and bays within which annual phytoplankton-related kills of penned salmon have occurred coincide with those areas where we found harmful *Chaetoceros* spp. to occur at the highest concentrations through the 0- to 10-m water column, as determined in both the spring and fall surveys (Fig. 2 and 5) as well as the site-specific sampling (Fig. 4). These data indicate that on several occasions during the spring and fall of 1988, the surface waters within these areas contained concentrations of *Chaetoceros* spp. which would be directly harmful to penned salmon. In addition, during October 1988, harmful concentrations of *Chaetoceros* spp. were present in the waters of Call Inlet (stations 39 and 40, Fig. 2 and 5).

Cochlan et al. (1990) have shown that in at least one region of the Strait of Georgia (the area immediately adjacent to the Fraser River plume), a summer subsurface chlorophyll maximum (SCM) occurs at a depth of approximately 12–14 m. Within this SCM, *Chaetoceros* spp. were a small proportion of the cell assemblage (the authors made no attempt to differentiate between harmful and harmless *Chaetoceros* spp.). Since SCMs have been found to occur in coastal seawaters elsewhere as well (Cullen and Eppley 1981; Cullen 1982; Kimor et al. 1987), it is possible that the concentrations of harmful *Chaetoceros* spp. which we report here for 0- to 10-m water columns along the British Columbia coast are underestimates of the concentrations of harmful *C. concavicornis* and *C. convolutus* through the 0- to 15-m water column.

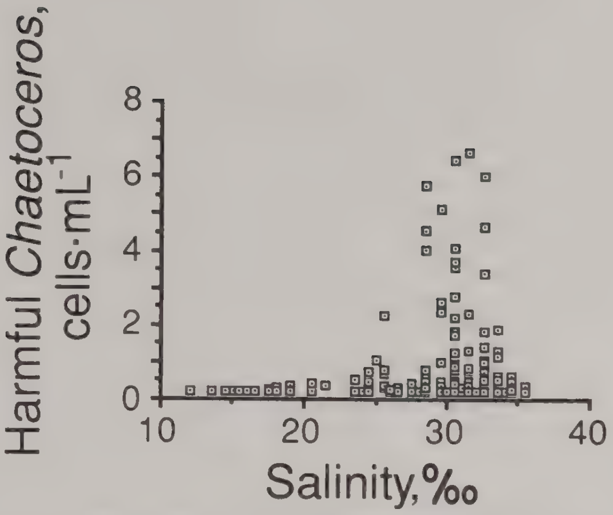

FIG. 6. Harmful *Chaetoceros* cell concentration versus salinity of surface waters removed from the southern, central, and northern coasts of British Columbia. A replot of the harmful *Chaetoceros* concentration data of Fig. 2 and 3 versus the salinity of each station.

The spring and fall bloom of diatoms, including *Chaetoceros* spp., are an annual phenomenon of temperate seawaters, including those of coastal British Columbia. In this respect, some of our data are consistent with what has been observed elsewhere, since water bodies such as Hotham Sound and Hoskyn Channel had a ''typically timed'' spring bloom (Fig. 4). However, at several locations such as Hardy Bay, Cordero Channel at Horn Point, and Frederick Arm (Fig. 4), the annual harmful *Chaetoceros* spp. ''spring'' bloom was delayed until the summer months (June through August).

In aquatic environments such as British Columbia coastal marine waters, it is difficult to determine which physical, chemical, and biological variables are important in controlling concentrations of harmful *Chaetoceros* spp. Our measurements from samples show that seawater salinity may be an important variable which influences the occurrence of these harmful phytoplankters. Figure 6 indicates that *C. concavicornis* and *C. convolutus* cells are not present in coastal British Columbia surface seawaters which are of salinity 17‰ or less. In addition, the lowered concentrations of these phytoplankters in seawaters between salinity 17 and approximately 27‰ imply that waters of these concentrations may not be optimum for their growth (Fig. 6), an implication which is supported by our unialgal culture experiments. Haigh and Taylor (1990) reported that the concentrations of *C. convolutus* in waters of the northern Strait of Georgia decreased with increased salinity. However, since surface waters of this region are normally 20–30‰ (Thomson

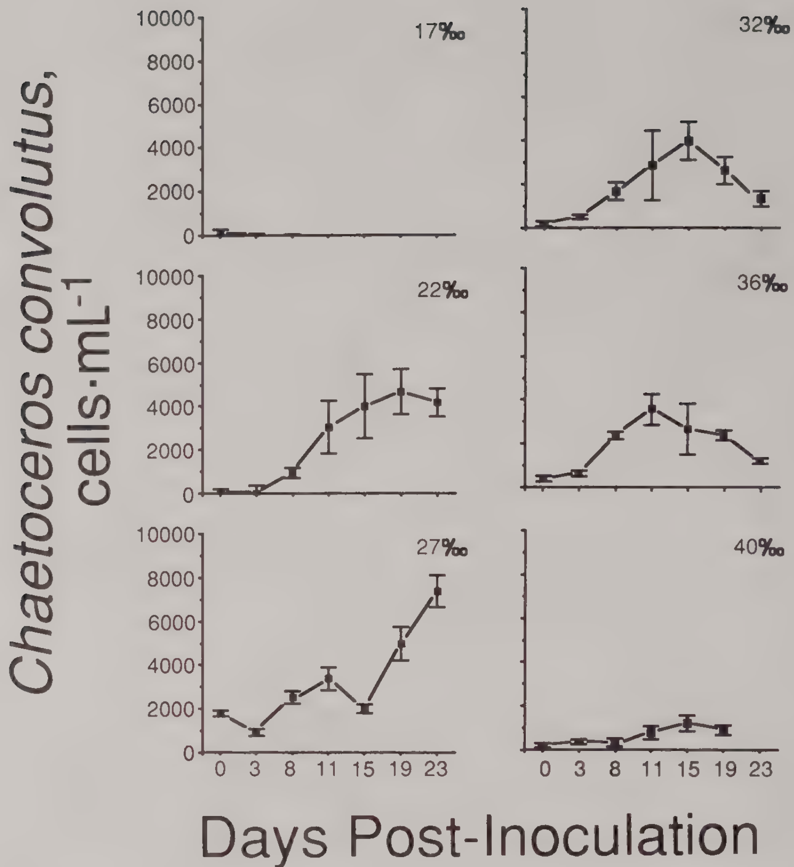

FIG. 7. Mean growth (±SD) of *C. convolutus* at 10–13°C in the medium of Harrison et al. (1980) at several salinities.

1981), our observations are not inconsistent with those of Haigh and Taylor (1990).

Brand (1984) in his study of the salinity tolerances of *Chaetoceros didymum, Chaetoceros pseudocrinitus, Chaetoceros lorenzianum*, and *Chaetoceros simplex* (the first two were isolated from coastal and the latter two from oceanic waters) found that the opimum salinity for growth of these cells was either 25 or 33‰. All four species grew at 15‰ (a salinity at which *C. concavicornis* and *C. convolutus* did not grow), but not at 5‰. The reason that these four nonharmful species of *Chaetoceros* are able to grow at a lower salinity than either *C. concavicornis* or *C. convolutus* is not known. These data indicate, however, that the inability of the harmful phytoplankters *C. concavicornis* and *C. convolutus* to grow at salinities of 17‰ and less is not necessarily characteristic of this genus.

Based upon the above results, semienclosed water bodies along the British Columbia coast where the surface seawater is diluted by freshwater and consequently is of <17‰ salinity should be free of these harmful *Chaetoceros* cells for at least a portion of the year. In this connection, the waters of Holberg Inlet and Indian Arm (Fig. 1) were investigated, since they are connected to Quatsino Sound and the Strait of Georgia, respectively, by sills at their seaward portion and each has a relatively high rainfall rate in the surrounding watershed. Harmful *Chaetoceros* cells were absent in the water at the Holberg Inlet site and almost completely absent in the water at the Indian Arm site during the period of this study. One would expect harmful *Chaetoceros* cells to be present to at least seed concentrations in Indian Arm, since this water body is contiguous to the Strait of Georgia which has relatively large seasonal concentrations of harmful *Chaetoceros* cells. Therefore, the absence of these harmful *Chaetoceros* cells except for two brief periods during the course of this study suggests that these phytoplankters may not be able to readily grow in the water of this inlet.

The salinity of the 0- to 10-m water column of Indian Arm varied from 22 to 31‰, with a mean over the year of 26.1‰. The surface water (0–30 m) salinity of Holberg Inlet varies between approximately 12 and 28‰ over an annual cycle, with the salinity exceeding 26‰ for much of the year (Drinkwater and Osborn 1975). Moreover, in each water body, the surface salinities were lowest between fall and spring, due mainly to freshwater runoff. Based upon these data, it would appear that lowered surface water salinities in the fall and spring may be a factor which limits growth of harmful *Chaetoceros* spp. in these two inlets.

In general, the surface waters of Johnstone Strait (except for Call inlet, stations 39 and 40, in October 1988) had significantly less harmful *Chaetoceros* spp. than the surface waters of the Strait of Georgia and its contiguous inlets and bays (Fig. 5), even though the surface waters were each within 1–2°C of each other during the sampling periods. One possibility is that the stratified waters of the Strait of Georgia and its contiguous inlets and bays may favour the growth of harmful *Chaetoceros* spp. rather than the turbulent and well-mixed surface waters of Johnstone Strait (see Thomson 1981).

The results of the present investigation indicate that although harmful *Chaetoceros* cells occur in seawater along the entire British Columbia coast, there are certain seawater bodies which are more prone to harmful *Chaetoceros* concentrations. These are seawater bodies which have salinities in excess of approximately 26‰. Waters least likely to contain these harmful *Chaetoceros* cells have salinities less than 22‰.

Our data indicate that the salinity of the water body may be a key determinant of the presence of harmful *Chaetoceros* cells. The turbulence of the water body may also be a determinant of the concentration of harmful *Chaetoceros*. However, other factors such as water temperature, nutrient content, and latitude as well as grazer activities are also likely important. The significance of these in the growth of harmful *Chaetoceros* spp. awaits further study.

The data presented here indicate that certain coastal seawaters appear to be more suitable for cultivation of penned salmon than others when the likelihood of harmful *Chaetoceros* blooms occurring is the sole consideration. In this regard, low-salinity waters such as those found in Holberg Inlet and Indian Arm as well as the mixed and turbulent water of Johnstone Strait appear to be suitable for cultivation of penned salmon. The waters of Jervis Inlet and the northern Strait of Georgia and its contiguous inlets appear to be much less suitable for cultivation of penned salmon.

Acknowledgements

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Ontogenetic Diet Shift in Lake Erie Age-0 Yellow Perch (*Perca flavescens*): A Size-Related Response to Zooplankton Density

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Wu, L., and D. A. Culver. 1992. Ontogenetic diet shift in Lake Erie age-0 yellow perch (*Perca flavescens*): a size-related response to zooplankton density. Can. J. Fish. Aquat. Sci. 49: 1932–1937.

Through field studies, we demonstrated that age-0 yellow perch (*Perca flavescens*) in western Lake Erie underwent an ontogenetic diet shift from exclusively zooplankton to mainly benthic prey during June through August. This shift coincided with a decline in zooplankton populations in the lake. Further, laboratory experiments on feeding ontogeny suggest that the diet shift was related to fish size. Yellow perch less than 30 mm total length did not consume benthic prey either during the feeding experiments or in the field studies, while fish greater than 50 mm did at low zooplankton densities. Interactions between fish size and zooplankton density, therefore, likely determine the ontogenetic diet shift in young-of-the-year yellow perch. Growth of yellow perch declined following the zooplankton decline. Reduced growth during this life stage may extend the period of vulnerability of yellow perch to its predators, thus reducing survival and, ultimately, recruitment.

Grâce à des travaux de terrain, nous avons montré que la perchaude (*Perca flavescens*) d'âge 0 dans l'ouest du lac Érié a connu un changement ontogénique dans son régime alimentaire, passant d'une diète constituée exclusivement de zooplancton à des proies principalement benthiques de juin à août. Ce changement a coïncidé avec un déclin des populations zooplanctoniques du lac. De plus, des expériences menées en laboratoire sur l'ontogenèse de l'alimentation semblent indiquer que le changement de régime était en rapport avec la taille des poissons. Les perchaudes de moins de 30 mm longueur totale ne consommaient pas de proies benthiques, ni pendant les expériences, ni dans les travaux de terrain, tandis que les poissons de plus de 50 mm le faisaient lorsque la densité du zooplancton était faible. Les interactions entre la taille des poissons et la densité du zooplancton semblent donc déterminer le changement ontogénique de régime alimentaire chez les jeunes perchaudes de l'année. La croissance des perchaudes baissait à la suite du déclin du zooplancton. La réduction de la croissance pendant ce stade biologique peut prolonger la période de vulnérabilité de la perchaude à ses prédateurs, ce qui doit réduire la survie et, en fin de compte, le recrutement.

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Many fish change their habitat use and diet as they grow, a phenomenon called an ontogenetic diet shift by Werner and Gilliam (1984). Mechanisms underlying the ontogenetic change in diet have been well demonstrated in systems where centrarchids dominate. For instance, in small Michigan lakes with a vegetated littoral zone, small bluegill (*Lepomis macrochirus*) switch from inshore macroinvertebrate feeding to offshore open-water zooplankton feeding as they grow to a size range of 70–100 mm total length (TL) (Mittelbach 1981; Werner and Hall 1988). Because predation risk from largemouth bass (*Micropterus salmoides*) decreases as bluegill grow larger, the move balances predation risk and growth, both of which are greater in offshore habitats. Thus, the diet shift in this case is a size-specific shift influenced by predation risk.

In many relatively large lakes in northern America, inshore habitat has little vegetation to serve as a refuge area for small young fishes. Inshore and offshore habitats often differ little in limnological character, except for the difference in depth. Many of these large lakes are dominated by percids such as walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*). Ontogenetic changes in habitat use by age-0 yellow perch relative to shore have been documented as either (1) horizontal offshore to nearshore migration associated with a diet change from offshore zooplankton to inshore macroinvertebrates in stratified lakes (Whiteside et al. 1985) or (2) vertical movement from pelagic to demersal habitats associated with a diet change from zooplankton to benthic prey (e.g. *Chironomus* spp.) in unstratified lakes (Lin 1975, cited in Post and McQueen 1988; Mills and Forney 1981, 1983). Whiteside et al. (1985) suggested that the littoral zone migration of larval yellow perch in Lake Itasca, Minnesota, is in response to a decrease in abundance of limnetic zooplankton. Similarly, Mills and Forney (1983) suggested that juvenile yellow perch in Lake Oneida, New York, change from pelagic zooplankton feeding to demersal benthic feeding in response to reduced zooplankton abundance in the pelagic habitat during midsummer. In contrast, Post and McQueen (1988) suggested that movement from nearshore to offshore by young-of-the-year yellow perch in Lake St. George, Ontario, is a genetically determined behavioral change. In this study, we examine the roles of zooplankton abundance and fish size on ontogenetic diet shifts in yellow perch.

Of the three basins in Lake Erie, the western basin is the most productive, serving as a nursery ground for many larval and juvenile fishes, so the biological factors influencing year-class strength of yellow perch there have potential significance for many other species as well. For example, the diet of age-0 yellow perch in the western basin can have a great impact on the species' growth and survival (Mills et al. 1987), as well as

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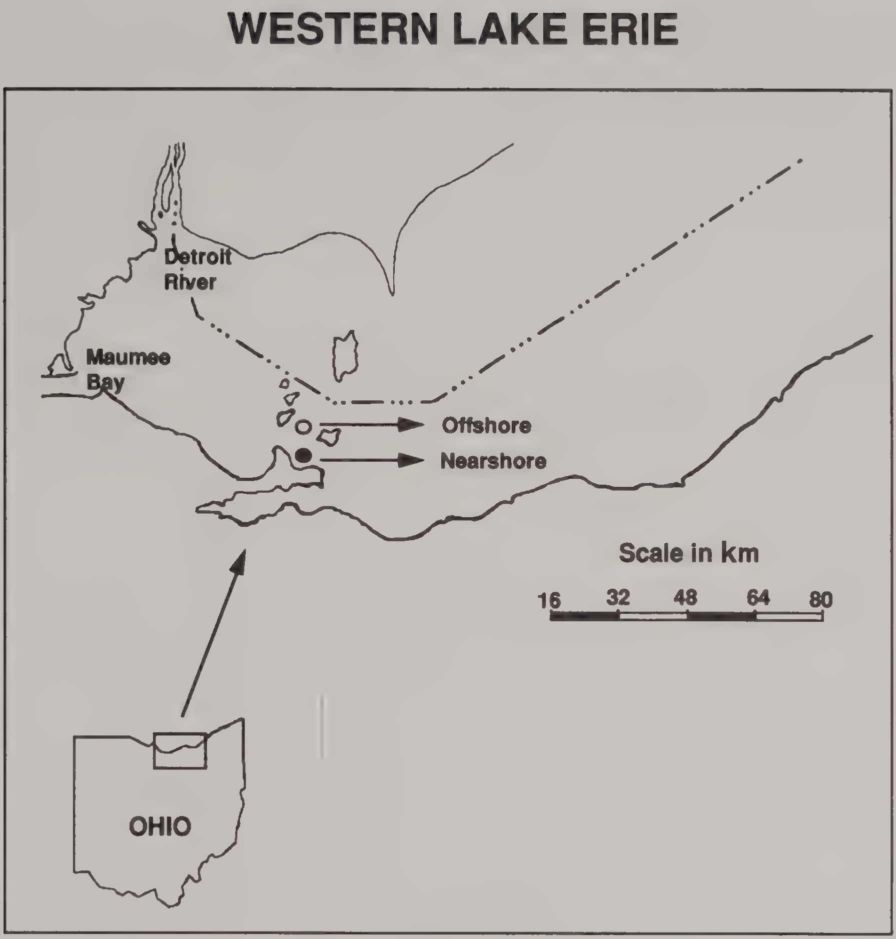

FIG. 1. Location of sampling sites in western Lake Erie.

the size of adult populations. Using a combination of field studies in western Lake Erie and laboratory experiments with age-0 yellow perch of selected sizes, we tested (1) whether an ontogenetic diet shift occurred in Lake Erie, (2) whether differences in the intensity of predation risk occurred that might influence ontogenetic shifts in nearshore and offshore habitats, and (3) whether growth of fish was reduced if yellow perch switched to feed on less optimal (high energetic costs associated with capture and ingestion of new prey) benthic prey (Mills et al. 1989). In addition, we tested whether the ontogenetic shift was a preprogrammed behavioral change associated solely with body size or whether zooplankton density was influential in initiating the switch. This last issue is important because it relates to the influence of zooplankton dynamics not only on diet and growth but also on the complex behavioral interactions that determine year-class strength in yellow perch. Because planktivorous larvae and juveniles of a diversity of other fish species (e.g., walleye, white perch (*Morone americana*), freshwater drum (*Aplodinotus grunniens*) occur in western Lake Erie at the same time, results for young-of-the-year yellow perch may reflect conditions for the young of the other dominant fish taxa in the lake.

Materials and Methods

Field Study Area

Zooplankton and age-0 yellow perch were sampled weekly in the western basin of Lake Erie at nearshore (41°33′53″N, 82°47′56″W) and offshore (41°38′67″N, 82°46′44″W) habitats (Fig. 1), 9.4 km apart, during June through August in 1988 and 1989. Sampling at this frequency is required to measure the rapid seasonal changes in zooplankton abundance. Neither the nearshore nor the offshore habitat had aquatic vegetation. We chose nearshore and offshore sampling because the distribution of zooplankton might differ in these habitats, consequently affecting diet and growth of yellow perch. The nearshore habitat was 6 m deep, receiving water mainly from the alongshore flow from Maumee Bay; the offshore habitat was 10 m deep, receiving general western basin flow primarily from the Detroit River (Burns 1985). Given these depths and the long fetch of the lake, the western basin did not stratify thermally. Surface and bottom temperatures rarely differed by >3°C in either habitat during late June through August in 1988 and 1989 (Fig. 2).

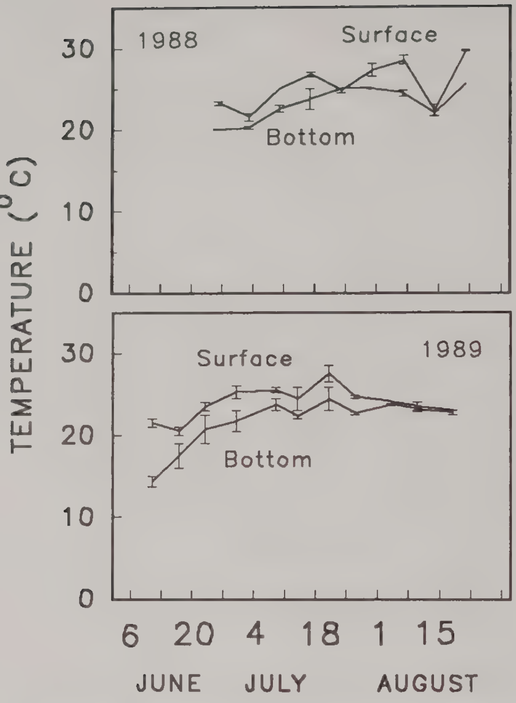

FIG. 2. Surface and bottom temperatures during June through August, 1988 and 1989, in western Lake Erie. Temperatures are means ± 1 SE ($N = 2$) measured at nearshore and offshore habitats.

Field Study

Zooplankton were collected between 13:30 and 16:00 weekly by duplicate samplings with a metered 0.5-m diameter net (112-μm mesh) towed vertically through the water column (down and up) at two sites (740 m apart) in both nearshore and offshore habitats. Zooplankton were preserved with a sugar–formalin (40 $g \cdot L^{-1}$) solution. In the laboratory, each zooplankton sample was diluted to 500–4000 mL. Two to three subsamples (5–15 mL) were then counted. At least 100 individuals of each abundant taxon (Balcer et al. 1984) were counted per sample.

Two 10-min otter trawls (3.66-m-diameter opening and 25-mm stretched mesh cod end) were conducted (at constant speeds) to collect age-0 yellow perch in both nearshore and offshore habitats. Adult piscivorous fish collected in the same trawls were identified and counted on the boat to determine predator densities and then returned to the lake. We used the distance travelled and the cross-sectional area of the trawl to estimate volume of water sampled, which was then used to estimate predator densities. Age-0 yellow perch were immediately chilled on ice and stored frozen for the diet and growth analysis. Diets were determined from 10 young-of-the-year juveniles selected randomly from weekly samples (June through August) in each habitat. Stomachs were removed and zooplankton and benthic organisms were identified and counted. Twenty individuals of each prey taxon were measured (nearest 0.02 mm). Cladocerans were measured from the top of the carapace to the base of the basal spine; copepods were measured from the top of the carapace to the base of the caudal rami. Head width and body length (if possible) were measured for *Chironomus* spp. Prey lengths were converted to individual dry weights (micrograms) using length–weight regressions (Dumont et al. 1975; Culver et al. 1985; G. G. Mittelbach, unpubl. data).

Total lengths were measured for all fish examined. Fish body and stomach content dry weights were determined after oven-drying for 48 and 24 h, respectively, at 80°C. Because switching from pelagic to benthic feeding can result in a reduction in growth of juvenile yellow perch (Crowder et al. 1987; Mills et al. 1989), we calculated yellow perch specific growth rate (r, Ricker 1973) as

$$r = \frac{[\ln(W_{t+1}) - \ln(W_t)]}{t}$$

where W_{t+1} and W_t = yellow perch body dry weight (grams) at two successive sampling dates and t = time between sample dates (days). Data were analyzed using repeated-measures ANOVA and Tukey's studentized test (GLM procedure, SAS Institute Inc. 1986).

Feeding Ontogeny Experiments

In 1990, we conducted feeding experiments at varied zooplankton densities but at constant larval chironomid density using three developmental stages of yellow perch larvae and juveniles (Table 1). With this design, we could determine whether age-0 yellow perch switch to benthic prey (chironomid larvae) in response to low zooplankton abundance or in relation to their body size.

In each set of experiments, young yellow perch were caught from St. Mary's State Fish Hatchery (160 km west of Columbus, OH) rearing ponds the afternoon before the experiments and acclimated in a 100-L aquarium overnight without feeding. For the experiments, we filled each aquarium with pond water strained through a 53-μm-mesh net. Zooplankton were collected from the pond and sized by rinsing them through a graded sieve series (291-, 425-, 500-, and 750-μm mesh size) before being added to the aquaria (Table 1). Various zooplankton densities (0–400 individuals$\cdot L^{-1}$) were established in the aquaria, while one chironomid (benthic prey) density ($1100 \cdot m^{-2}$) was used. These zooplankton densities are within total zooplankton densities observed in western Lake Erie (Fig. 3). Chironomids were raised in aquarium cultures.

At the beginning of each experiment, one fish was introduced into each aquarium. After 2 h, fish were preserved with 10% formalin and their stomach contents analyzed to determine the number of zooplankton and chironomids consumed. Zooplankton remaining were recovered by filtering the aquarium water through a 32- or 53-μm-mesh net. The initial zooplankton density was estimated from the sum of the zooplankton remaining and those consumed.

Behavioral Experiments

To determine where yellow perch captured chironomids, we observed yellow perch feeding positions from about 4 m away using binoculars. In these experiments, the aquarium, which contained pond zooplankton and laboratory-raised chironomids, was marked every 5 cm with colored bands from surface to bottom to facilitate the location of fish and chironomids. At least five yellow perch from the large size-class (Table 1, mean TL = 53.70 mm) were placed in the aquarium. We continually recorded the position of (1) yellow perch and (2) chironomids and (3) the location at which chironomids were captured.

TABLE 1. Experimental conditions for feeding trials conducted from May through June 1990 in St. Mary's State Fish Hatchery, Ohio.

	Fish size-class		
	Small	Medium	Large
Experimental period	May 15–19	May 29–31	June 21–23
Aquarium size (cm)	30 × 15 × 20	30 × 15 × 20	50 × 25 × 30
Number of trials	45	51	29
Zooplankton density range (no.·L^{-1})	0–150	0–120	0–450
Zooplankton size (μm)	291–425	500–750	500–750
Chironomid size (mm) (mean (± SD))			
Head width	0.38 (± 0.05)	0.22 (± 0.06)	0.47 (± 0.07)
Body length	3.47 (± 0.69)	2.38 (± 0.49)	5.27 (± 1.23)
Fish gape width (mm)	1.41 (± 0.22)	2.35 (± 0.69)	4.63 (± 0.69)
Fish total length (mm)	15.09 (± 0.81)	26.86 (± 1.97)	53.70 (± 2.29)

Results

Field Pattern

Zooplankton dynamics

Total zooplankton abundance in the lake fluctuated during both 1988 and 1989; peak abundance occurred in June, followed by a continuous decline until mid-July (Fig. 3A). This decline in abundance (1988: June 22 – July 27; 1989: June 20 – July 24) did not differ between nearshore and offshore habitats (repeated-measures ANOVA, habitat effect $F_{1,4} = 3.30$, $P = 0.14$). All results were thereafter presented with the nearshore and offshore data combined. Cladoceran populations fluctuated quite similarly to total zooplankton. They disappeared in August 1988 (Fig. 3B), although a small recovery did occur in August 1989. Copepods did not vary as dramatically as cladocerans (Fig. 3C). Because rotifers and nauplii were never eaten by age-0 yellow perch in western Lake Erie, they were not included in the zooplankton and copepod analyses from the lake.

Yellow perch diet and growth

Age-0 yellow perch collected in both years consumed zooplankton exclusively (Fig. 4) before the decline in zooplankton abundance in western Lake Erie (Fig. 3). The onset of consumption of benthic organisms coincided with declining zooplankton abundance in the lake in both years (Fig. 4). After the crash in zooplankton populations, stomach contents consisted mainly of benthic prey (micrograms per fish).

Growth (r) was reduced (Tukey's studentized test) following the zooplankton decline in both 1988 (before: mean $r = 0.074$, $N = 12$; after: mean $r = 0.040$, $N = 12$; $P = 0.025$) and 1989 (before: mean $r = 0.101$, $N = 16$; after: mean $r = 0.059$, $N = 9$; $P = 0.026$).

Predator distribution

The adult fish community in 1988 and 1989 was dominated by yellow perch and white perch but also included walleye and freshwater drum. Adult yellow perch (age 2+ and older) are predators and 5–20% of their diet in western Lake Erie consists of small yellow perch (Knight et al. 1984). Predator density never exceeded 0.01 fish·m^{-3} (Fig. 5), except for one sampling day in the offshore area in 1988. Further, predator density did not differ between nearshore and offshore (repeated-measures ANOVA, habitat effect) in either 1988 ($P = 0.059$) or 1989 ($P = 0.84$).

Feeding Ontogeny Experiments

Yellow perch <30 mm TL (small and medium sizes in Table 1) rarely consumed chironomids, even when zooplankton densities were very low (close to zero). Yellow perch <30 mm TL were capable of ingesting the chironomids, based on a comparison of fish mouth gape and chironomid head width (Table 1). Only large yellow perch (mean TL = 53.70 mm) fed on benthic prey. The number of chironomids eaten increased as zooplankton density decreased ($P < 0.05$, Jonckheere–Terpstra ordered test; Hollander and Wolfe 1973) (Fig. 6).

Behavior

Yellow perch (large size in Table 1) in the observation aquarium searched for and directly pursued chironomids before ingestion. Furthermore, yellow perch captured chironomids from either the bottom of the aquarium or a region less than 10 cm from the bottom. All chironomids (except for two individuals) settled to and moved along the bottom of the aquarium during the observations.

Discussion

A growing larva generally exhibits three dynamic behaviors to fulfill its increasing metabolic demands: (1) eating more or larger individuals, (2) eating new prey taxa, and (3) modifying its feeding strategy during ontogeny (Hartman 1983). In addition to increasing the number of prey eaten, yellow perch in western Lake Erie switched from feeding on only zooplankton to feeding on mainly benthic prey during their first summer of life. The diet shift in yellow perch occurred during the period of zooplankton decline, suggesting that a decreasing zooplankton density could cause increased feeding on benthic prey. A similar shift to benthic prey occurs among juvenile yellow perch in Oneida Lake, New York, after *Daphnia* disappear from the pelagic zone (Mills and Forney 1983).

However, we demonstrated that reduced zooplankton density (<50·L^{-1}) only caused yellow perch of large size (mean TL = 53.70 mm) to switch to feeding on benthic prey. Field results also revealed that yellow perch in western Lake Erie were greater than 30 mm TL (Fig. 7) when they switched from feeding on zooplankton alone to feeding on benthic prey and that the switch occurred at a concentration of suitable zooplankton prey of about 50·L^{-1} (Fig. 3A). Similarly, yellow perch in Lake Itasca, Minnesota, change from offshore zooplankton feeding to nearshore macroinvertebrate feeding at 25 mm TL (Whiteside et al. 1985). Post and McQueen (1988) also reported that the offshore to nearshore transition of yellow perch in Lake St. George, Ontario, occurred at 30 mm TL but failed to correlate the move with changes in prey abundance in the lake.

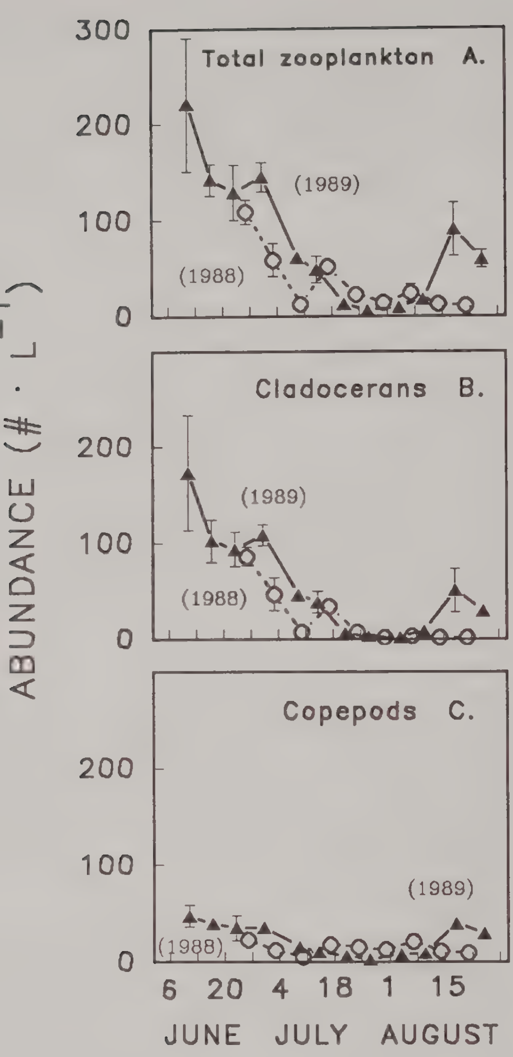

FIG. 3. Zooplankton abundances during 1988 (open circles) and 1989 (solid triangles) in the western basin of Lake Erie. Values are means ± 1 SE from four vertical haul samples collected at both nearshore and offshore habitats weekly during June through August.

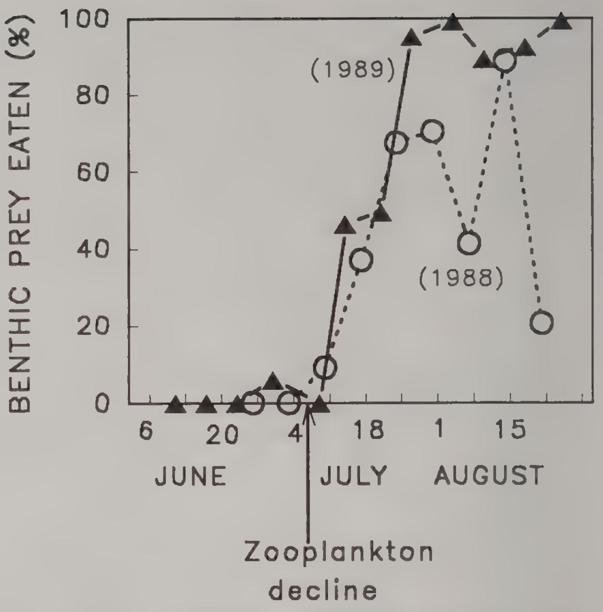

FIG. 4. Percent by weight of benthic prey (*Chironomus, Alona*, Ostracoda) in stomach contents of age-0 yellow perch from western lake Erie, 1988 (open circles) and 1989 (solid triangles). There were no differences between fish collected from nearshore and offshore habitats.

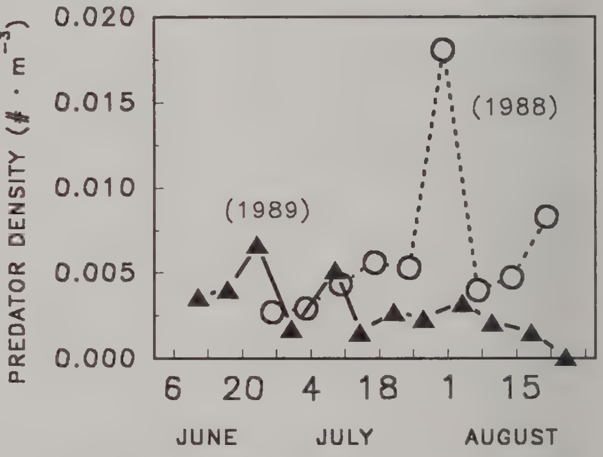

FIG. 5. Density of potential predators (age 2+ and older yellow perch and walleye) during June through August in 1988 (open circles) and 1989 (solid triangles), estimated from two otter trawls conducted weekly in both nearshore and offshore habitats in the western basin of Lake Erie.

Size-related changes in food types as fish grow have also been associated with the important effect of fish size in determining habitat use and feeding ability (e.g. mouth gape size) and their vulnerability to predators (Mittelbach 1981; Werner 1986). While it may have occurred, we have little evidence of nearshore or offshore movement of young-of-the-year yellow perch. In western Lake Erie, small yellow perch were physically capable of eating benthic organisms, including chironomids, and there was little seasonal change in predator density or difference between the nearshore and offshore habitats. Furthermore, 95% of the time, the predator densities in western Lake Erie were less than 0.01 fish·m^{-3}, the level at which Turner and Mittelbach (1990) demonstrated an effect of largemouth bass on habitat use by young bluegill. Thus, if predator-mediated shifts in resource and/or habitat use by age-0 fish (Turner and Mittelbach 1990) are less important in western Lake Erie than in lakes with complex littoral zones, it may be because pelagic piscivores are less concentrated in one place.

Combined field and laboratory results suggest that the ontogenetic diet shift from zooplankton to benthic prey in age-0 yellow perch was a result of size-related change during fish development *and* low zooplankton density in the lake. Because the western basin is usually homothermal and has little vegetated inshore habitat and because the dynamics of zooplankton and the distribution of predators did not differ between nearshore and offshore habitats, the movement of age-0 yellow perch from pelagic to benthic zones is likely in both nearshore

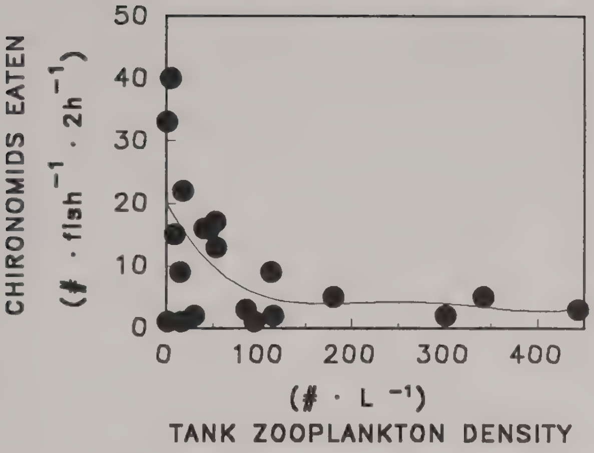

FIG. 6. Influence of zooplankton density on the number of benthic prey (chironomids) consumed by yellow perch (mean TL = 53.70 mm) during 2-h feeding experiments during June 21–23, 1990. Number of trials = 29.

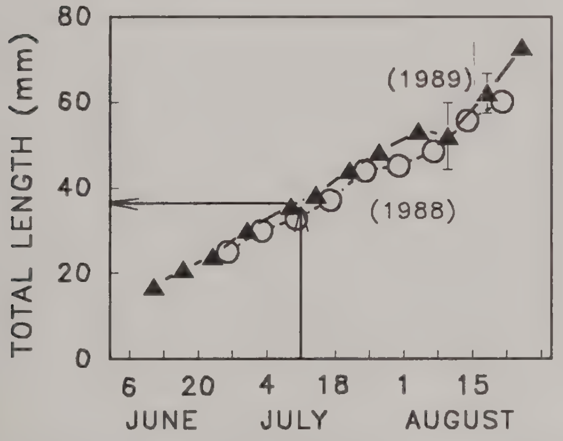

FIG. 7. Total length of age-0 yellow perch collected weekly during June through August in nearshore and offshore habitats in western Lake Erie, 1988 (open circles) and 1989 (solid triangles). Arrows indicate the time of the diet shift and the corresponding fish total length. Vertical bars are 95% confidence intervals of the means. For most dates, they do not extend beyond the symbols marking the means.

and offshore habitats. Growth of yellow perch in western Lake Erie was reduced in late summer when yellow perch ate benthic organisms after the zooplankton population crashed relative to early summer when zooplankton were abundant. Reduced growth may extend the period of vulnerability of young yellow perch to piscivorous predation (Crowder et al. 1987; Rice et al. 1987) and thus influence age-0 yellow perch survival and reduce recruitment. Therefore, understanding the relative importance of zooplankton abundance, fish size, and size-specific behavioral changes in determining diet during development is of significance in predicting the growth and survival of age-0 fishes.

Acknowledgements

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Geochemistry of Quebec North Shore Salmon Rivers during Snowmelt: Organic Acid Pulse and Aluminum Mobilization

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Campbell, P. G. C., H. J. Hansen, B. Dubreuil, and W. O. Nelson. 1992. Geochemistry of Quebec North Shore salmon rivers during snowmelt: organic acid pulse and aluminum mobilization. Can. J. Fish. Aquat. Sci. 49: 1938–1952.

The episodic acidification of three salmon rivers on the Quebec North Shore during spring snowmelt is shown to result mainly from increased inputs of *organic* acids rather than strong mineral acids. Appreciable mobilization of Al occurs during the spring pH depression, accompanied by marked changes in Al speciation. Saturation indices, calculated for each river with respect to microcrystalline gibbsite ($K_{so} = 10^{-9.35}$), approach saturation for samples collected before or after the snowmelt period, but during maximum discharge, marked undersaturation is observed. Seasonal differences in Al – dissolved organic carbon (DOC) interactions were also noted. Outside the snowmelt period, a linear logarithmic model proved successful in predicting concentrations of monomeric organic Al ($[\text{Al-org}] = 3.55 \cdot 10^{-3} \cdot \{\text{Al}^{3+}\}^{0.129} \cdot \{\text{H}^+\}^{0} \cdot [\text{DOC}]^{0.91}$; $n = 19$, $r^2 = 0.92$), but during snowmelt, this model greatly overestimated [Al-org]. The failure of the model during this critical period is attributed to the presence in the spring DOC peak of an appreciable nonhumic (and non-Al-binding) component. Although concentrations of monomeric inorganic Al (notably fluoro–Al complexes) increase during the spring pH depression, levels do not appear to attain the thresholds for Al and H^+ toxicity reported for juvenile Atlantic salmon (*Salmo salar*).

Nous montrons que l'acidification épisodique de trois rivières à saumon de la Côte-Nord du Québec pendant la fonte des neiges résulte principalement d'une hausse des apports d'acides organiques, plutôt que d'acides minéraux forts. On observe pendant la baisse printanière du pH une mobilisation notable d'Al, accompagnée par des changements marqués dans la proportion des espèces d'Al. Les indices de saturation, calculés pour chaque cours d'eau en ce qui concerne la gibbsite microcristalline ($K_{so} = 10^{-9,35}$), approchent la saturation pour les échantillons prélevés avant ou après la période de la fonte des neiges, mais on observe pendant la période de débit maximal une sous-saturation marquée. On a aussi noté des différences saisonnières dans les interactions Al – carbone organique dissous (COD). En dehors de la fonte des neiges, un modèle logarithmique linéaire a pu prédire avec succès les concentrations d'Al organique monomère ($[\text{Al-org}] = 3{,}55 \cdot 10^{-3} \cdot \{\text{Al}^{+3}\}^{0{,}129} \cdot \{\text{H}^+\}^{0} \cdot [\text{COD}]^{0{,}91}$; $n = 19$, $r^2 = 0{,}92$), mais pendant la fonte des neiges ce modèle surestimait fortement [Al-org]. Les mauvais résultats donnés par le modèle pendant cette période critique sont attribués à la présence dans le pic printanier de COD d'une importance composante non humique (et ne liant pas Al). Bien que les concentrations d'Al inorganique monomère (notamment les complexes fluoro–Al) augmentent pendant la baisse printanière du pH, les teneurs ne semblent pas atteindre les seuils de toxicité d'Al et de H^+ signalés pour les saumons de l'Atlantique (*Salmo salar*) juvéniles.

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Of the known geochemical responses to environmental acidification, probably the best documented is the mobilization of Al from terrestrial to aquatic environments (for reviews, see Driscoll and Schecher 1988; Nelson and Campbell 1991). This mobilization is often episodic in nature, associated with pH depressions occurring during spring snowmelt or specific storm events (Driscoll et al. 1980; Henriksen et al. 1984; LaZerte and Dillon 1984; Borg 1986; McAvoy 1989). The resulting elevated Al concentrations, together with the low pH, can be harmful to a variety of aquatic organisms (NRCC 1986), notably salmonid fish (Cronan and Schofield 1979; Henriksen et al. 1984; Holtze and Hutchinson 1989). In a field setting, the biological consequences of this episodic mobilization will depend on the duration of the episode, the Al concentrations attained, the forms present (Al speciation), and the prevailing physicochemical conditions (e.g. $[H^+]$, $[Ca^{2+}]$).

The present study was carried out on the Quebec North Shore, an area some 200–400 km wide that lies to the north of the Gulf of Saint Lawrence between Tadoussac and Blanc-Sablon (Fig. 1, Quebec hydrographic region 07). Many Atlantic salmon rivers can be found in this region, draining north to south off the Precambrian Shield; since 1981 the Canadian Department of Fisheries and Oceans (DFO) has maintained a monitoring network of stations situated in the downstream freshwater reaches of 21 of these rivers. In an analysis of the 1982 and 1983 bimonthly results from this network, Walsh et al. (1987) reported that pH and alkalinity were consistently lower in samples that had been collected during the spring snowmelt period.

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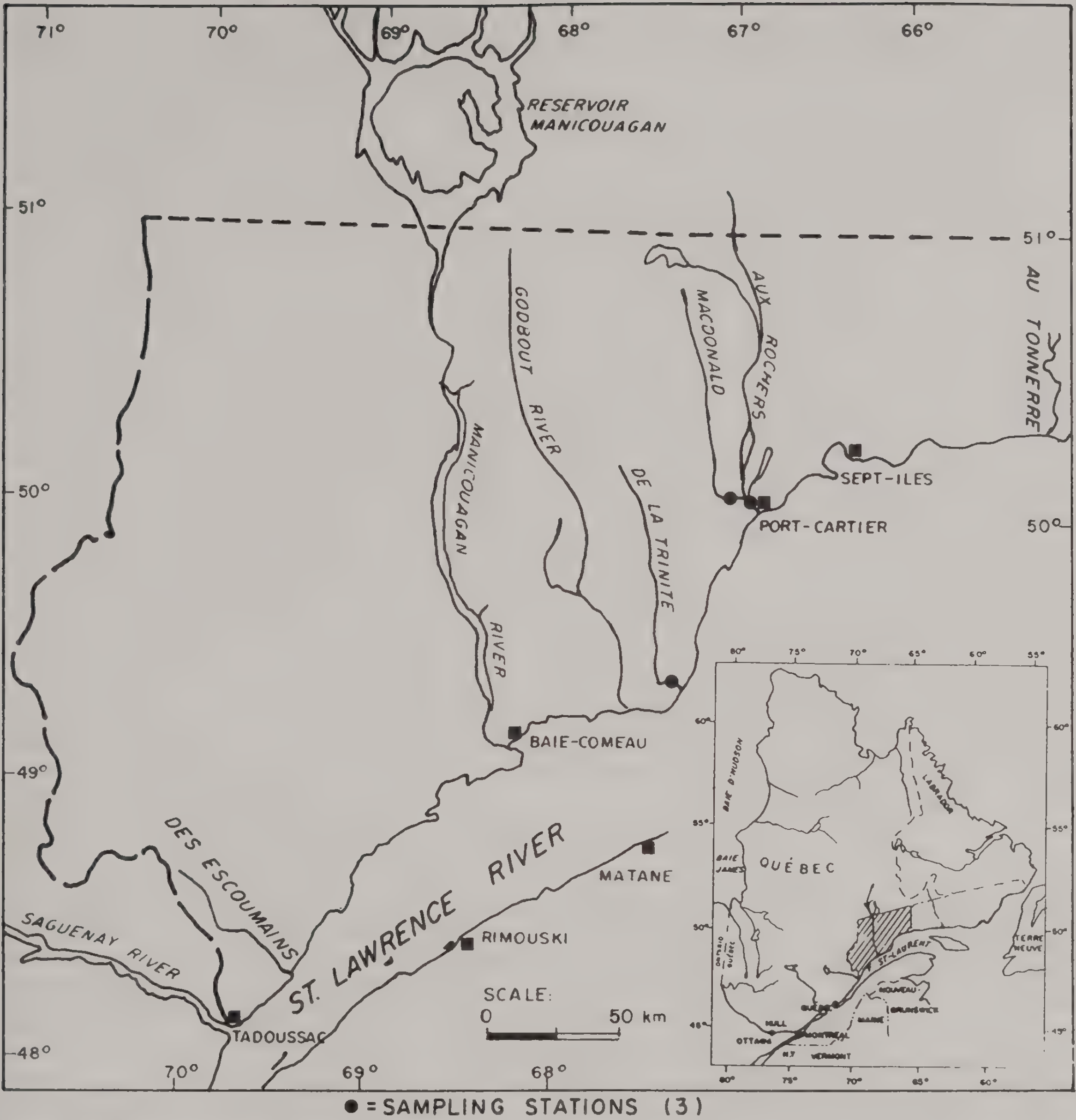
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DE LA TRINITE
MANICOUAGAN RIVER
SEPT-ILES
PORT-CARTIER
50°
50°
BAIE-COMEAU
49°
DES ESCOUMAINS
SAGUENAY RIVER
ST. LAWRENCE RIVER
MATANE
RIMOUSKI
TADOUSSAC
SCALE:
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70°
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68°
BAIE D'HUDSON
LABRADOR
BAIE JAMES
QUÉBEC
ONTARIO QUÉBEC
ST-LAURENT
TERRE NEUVE
HULL
OTTAWA
MONTREAL
N.Y.
VERMONT
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FIG. 1. Map of the Quebec North Shore showing the De la Trinité, Aux Rochers, and McDonald rivers and the location of the three sampling sites (●).

Given this combination of a region inherently sensitive to acid deposition (Environment Canada 1985; Dupont 1991), and the presence of a valuable fish species known to be affected by low pH and elevated Al concentrations (Henriksen et al. 1984; Skogheim and Rosseland 1986) and reported to be in decline (Caron 1990), we undertook a joint study with DFO of the geochemistry of several representative salmon rivers on the Quebec North Shore. The objectives of the study were to (i) substantiate earlier evidence for spring pH depression in these rivers and identify the causes, (ii) determine the concentrations and speciation of Al during this critical period, and (iii) compare these conditions with those reported to have affected Atlantic salmon (*Salmo salar*) stocks elsewhere.

Materials and Methods

Description of the Study Area

The three sampling sites lie in the catchments of the Aux Rochers and De la Trinité rivers, which discharge into the Gulf of Saint Lawrence between the towns of Baie-Comeau and Sept-Îles (Fig. 1). These catchments are underlain by granitic bedrock belonging to the Grenville group; surface deposits are sparse and noncalcareous, their composition resembling that of the granitic parent material. Shallow soils (25–100 cm) of glacial origin are found throughout the North Shore region, with ferro-humic and humo-ferric podzols predominating; wetlands are also abundant (Ducruc 1985). The catchments exhibit low relief and are virtually undisturbed, with few roads and little human activity. The vegetative cover is dominated by black and white spruce (*Picea marina* and *P. glauca*) and by balsam fir (*Abies balsamea*).

The mean annual temperature in the region is 1°C, with a growing season of 105 d (i.e. period with mean daily temperature >5.6°C; Naiman et al. 1987). The total annual precipitation of 102–132 cm is evenly distributed throughout the year, with mean monthly values ranging from 8.0 cm in April to 10.6 cm in September and December. The mean annual pH of the precipitation ranges from 4.2 to 5.0 (Barrie and Sirois 1982; Grimard 1984, 1985); estimates of sulphate wet deposition range from 10 to 15 $kg \cdot ha^{-1} \cdot yr^{-1}$ (J. Dupont, Environnement Québec, Direction de la qualité des cours d'eau, Ste-Foy (Québec), pers. comm.). Precipitation from October to April occurs mostly as snow, accounting for 35–40% of the total (Ferland and Gagnon 1967). Spring snowmelt dominates the annual hydrological cycle, with about 50% of the annual discharge occurring in April and May. Minimum flows generally occur in January and February, interspersed with occasional short thawing periods; daily discharges during these winter months are normally <5% of the maximum daily flows encountered during snowmelt.

Surface water quality in the North Shore region reflects the abundant precipitation, the cool climate, and the resistance of the surface deposits and bedrock to weathering. Mean annual conductivities for the 21 rivers monitored by DFO are low and show relatively little spatial variability (13–22 μS/cm, overall mean of 17 ± 3 μS/cm). Mean annual alkalinities for these same rivers are also low but somewhat more variable, ranging from 9 to 136 μequiv/L with an overall mean of 56 ± 32 μequiv/L (Walsh et al. 1987). Nutrients are in short supply and the surface waters of the entire region have been described as highly oligotrophic (Duthie and Ostrofsky 1975).

Sampling

Water samples (1.5 L) were collected by DFO personnel in acid-washed (HCl, 50% v/v) polyethylene bottles at two sites located in the lower reaches of the De la Trinité and Aux Rochers rivers and from one site on the McDonald River, a major tributary of the aux Rochers (catchment areas upstream from the sampling sites were 562 (TR), 4170 (AR), and 682 (McD) km^2, respectively). The three sampling sites corresponded to previously identified salmon spawning beds. The sampling period extended from February to June 1986 with a sampling frequency that varied from bimonthly, during winter low flows, to two to three times per week during the critical spring runoff period. Samples were transported in coolers (4°C) to Quebec City for analysis.

Additional water samples (large volume, 200 L) were collected from the De la Trinité River in March, May, and August 1988; these samples were treated with XAD resins to isolate the fulvic and humic acid components of the dissolved organic matter (DOM) present (see below).

Reagents

Deionized water for the preparation of stock solutions was obtained from a Milli-Q3RO/Milli-Q2 system (Millipore Corp.; resistivity >15 Mohm/cm). To minimize Al contamination, laboratory manipulations were carried out in polyethylene or polypropylene containers. This labware was soaked in dilute nitric acid (20% v/v, 24 h) and carefully rinsed with deionized water before use. Reagents used in this study were of analytical grade or better.

Analyses

Calcium, Mg, Na, K, Fe, and Mn were determined on acidified samples (2 μL ultrapure HNO_3/mL) by flame atomic absorption spectrophotometry. Chloride, F, and SO_4 were measured by ion chromatography, NO_3 was determined by hydrazine reduction, and alkalinity was evaluated by Gran titration. These analyses were carried out by the DFO regional laboratory (Quebec City); standard QA/QC procedures were employed, and overall laboratory performance in interlaboratory comparison exercises was consistently satisfactory.

pH was measured with a glass electrode calibrated against standard buffer solutions; NaCl was added to each sample (150 μL of 1.0 M NaCl per 15-mL sample) to raise the ionic strength and minimize problems with electrode junction potential. Samples for dissolved organic carbon (DOC) analysis were filtered (0.4-μm Nuclepore filters) and photooxidized; the inorganic C produced was determined by automated dialysis against a standard solution of NaOH followed by conductimetry.

Concentrations of organic acid anions ($RCOO^-$) were estimated by charge balance (Cronan et al. 1978). As an internal check, concentrations were also calculated on the basis of an empirical equation involving DOC concentrations ("*n*" milliequivalents of $-COO^-$ per milligram of organic C) and an algorithm expressing the ionization behaviour of these carboxyl groups over the pH range 3–7 (i.e. the Oliver et al. (1983) model, as recalibrated recently for the Quebec North Shore region by Wilkinson et al. (1992)). Agreement between the two methods was reasonable ($[\text{anion deficit}]_{\text{measured}} = 0.84\,[RCOO^-]_{\text{calc}} + 17$; $r^2 = 0.62$, $n = 47$, units = microequivalents per litre) and the same pattern of change was observed for both estimates during the spring runoff period.

The Al speciation measurements involved an initial filtration step (0.4-μm polycarbonate filters; Nuclepore) to distinguish operationally between particulate and dissolved Al; aliquots of both the original sample and the filtrate were acidified (2 μL HNO_3/mL) and Al was determined by atomic absorption spectrophotometry with electrothermal atomization (AA-graphite tube). An automated pyrocatechol violet (PCV) spectrophotometric procedure was used to determine labile forms of Al in a portion of the remaining (unacidified) filtrate. One portion of the filtrate was analyzed directly; another was passed through a cation-exchange resin and then subjected to the same PCV colorimetric analysis (Rogeberg and Henriksen 1985). This analytical approach yields four Al values: $[Al]_t$ (total Al: unfiltered sample, acidified, AA analysis); $[Al]_d$ (dissolved Al: filtered sample, acidified, AA analysis); $[Al]_{pcv}$ (dissolved monomeric Al, both inorganic and organic forms: filtered sample, PCV analysis); and $[Al]_{nex}$ (nonexchangeable monomeric Al, presumably organic bound: filtered sample, after resin treatment, PCV analysis). Inorganic monomeric Al was estimated by difference ($[Al]_{pcv} - [Al]_{nex}$); the distribution of the various inorganic complexes within this pool was calculated from the results for dissolved inorganic monomeric Al, Ca, Mg, pH, total F, and total SO_4 with the ALCHEMI chemical equilibrium model (Schecher and Driscoll 1987). The equilibrium constants were adjusted within the model to take into account variations in ambient water temperature (0.1–10°C).

Isolation of Fulvic/Humic Acids

To test for seasonal variations in the metal complexing properties of the DOM, 200-L water samples were collected from the De la Trinité River before, during, and after spring runoff in 1988. These samples were filtered under nitrogen pressure through polycarbonate membranes (0.4-μm pore size, 142-mm diameter; Nuclepore) to remove any suspended matter; progressive obstruction of the filters was a problem and numerous filter changes were needed to process each 200-L sample.

The preparative isolation of the aquatic fulvic and humic acids closely followed the procedure described by Thurman and Malcolm (1981). The filtrates were acidified (pH 2, ultrapure HCl) and pumped through a carefully cleaned Amberlite XAD-8 macroreticular resin (1.2-L bed volume, flow rate 9 L/h). The hydrophobic acids adsorbed onto the resin were eluted in the reverse direction with 0.1 M NaOH (1.8 L, flow rate 3 L/h). The eluate was reacidified to pH 2, recycled through a smaller XAD-8 column (bed volume 60 mL), and again desorbed with 0.1 M NaOH (108 mL). The reconcentrate was adjusted to a DOC concentration of 500 mg/L, acidified to pH 1.0 with concentrated HCl, and allowed to stand for 24 h. The precipitate (humic acid) was collected by centrifugation, washed free of chloride, taken up in 0.1 M NaOH, and diluted to a DOC concentration of about 100 mg/L. The fulvic acid fraction, present by definition in the supernatant after centrifugation, was readsorbed onto XAD-8, washed free of excess NaCl with deionized water, and back-eluted with 0.1 M NaOH. The sodium fulvate and sodium humate solutions were then passed through a strong cation exchange resin in the hydrogen-saturated form to convert the sodium salts to the free-acid forms. Finally, the hydrogen (proton)-saturated acids were isolated as amorphous brown solids by lyophilization.

Titration of the Isolated Fulvic/Humic Acids with Al

The affinity of the fulvic and humic acid samples for Al and their complexation capacity for this element were determined by batch titration at pH 5. Batch titration was chosen so as to allow long equilibration times for each point on the titration curve. For each sample, a stock solution (2 L) of the following composition was prepared: fulvic or humic acid, 10 mg/L; $NaHCO_3$, 12 mg/L; $CaSO_4 \cdot 2H_2O$, 7.5 mg/L; $MgSO_4$, 7.5 mg/L; KCl, 0.5 mg/L. This solution was then adjusted to pH 5.1 with HCl (final concentrations (microequivalents per litre): Ca, 88; Mg, 124; Na, 145; SO_4, 214; Cl, 152). The solution was subdivided into 48 separate aliquots (40 mL) and to each such portion was added an aliquot (0–1000 μL) of a standard Al solution as titrant (pH 4.1, [Al] = 380 μM); a complementary aliquot (1000–0 μL) of acidified deionized water (pH 4.1) was also added to maintain constant final conditions (volume = 41 mL, pH = 5.0). Final Al concentrations ranged from 0 to 9.3 μM; 24 separate concentrations were tested in duplicate.

After an equilibration of at least 48 h at room temperature, the concentrations of free and bound Al were determined in each of the individual Al – fulvic/humic acid solutions. These Al speciation measurements were performed as described earlier for the river water samples.

Results

Snowmelt Hydrology

Although the De la Trinité, Aux Rochers, and McDonald rivers were not gauged, discharge values are available for several other rivers to the west (Des Escoumains; Godbout) and east (Au Tonnerre) of the study sites. The 1986 spring hydrographs for these three rivers are very similar, with peak discharge occurring more or less synchronously (Fig. 2). Given its proximity to the three sampling sites (immediately to the west of the De la Trinité catchment, see Fig. 1), we have used the Godbout River as a hydrological surrogate.

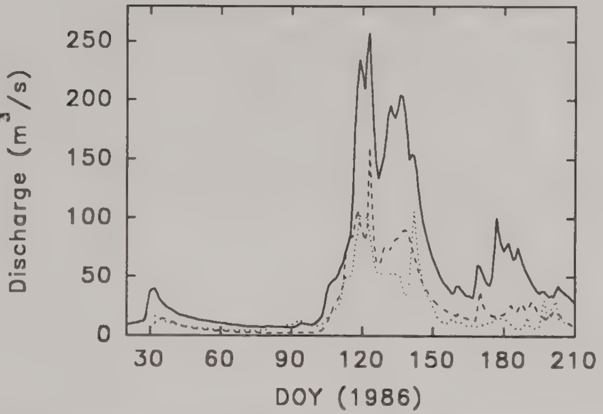

FIG. 2. Hydrographs for the Godbout (solid curve), Des Escoumains (dotted curve), and Au Tonnerre (broken curve) rivers for January to July 1986. All three rivers are shown in Fig. 1; the Godbout River is situated immediately to the west of the study area. Discharge was monitored at gauging stations located just upstream from the confluence of the rivers with the Gulf of St. Lawrence (catchment areas (km^2): Godbout = 1570; Des Escoumains = 793; Au Tonnerre = 676).

A slight thaw occurred in the study region just before the initial sampling (2 February 1986), causing a small increase in river flow, but thereafter, ambient temperatures remained below the freezing point until mid-April; river flows were very stable during this winter period (4–13 $L \cdot s^{-1} \cdot km^{-2}$). Spring runoff in the study area occurred from mid-April to late May; river flows peaked twice during this period (29 April – 2 May; 12–16 May), attaining values of 159 and 127 $L \cdot s^{-1} \cdot km^{-2}$, respectively, and then declined smoothly until mid-June.

Water Chemistry Changes during Snowmelt

Temporal trends in water quality closely followed the seasonal changes in river discharge. During the winter low-flow period (days 30–90), river water chemistry was relatively stable; base cation concentrations tended to increase progressively as discharge approached baseline values, but such variables as pH, DOC, SO_4, and NO_3 showed little fluctuation. With the onset of snowmelt, however, all water quality parameters responded: most solutes decreased in concentration, as is normally predicted for inorganic constituents from flow path and/or kinetic arguments, but several variables (notably $[H^+]$, water colour, and [DOC]) demonstrated the opposite trend and attained maximum values during the peak flows. The temporal variability of six key parameters (pH, alkalinity, Ca, SO_4, NO_3, and DOC) is illustrated for each site (Fig. 3–5).

All three rivers experienced marked pH and alkalinity depressions during the snowmelt period. For each river, plots of pH as a function of time exhibit two minima, corresponding closely to the two peaks in river flow (days 120 and 135). The lowest pH values were observed during the first flood peak: McDonald, pH 5.3, alkalinity 14 μequiv/L; Aux Rochers, pH 4.9, alkalinity −2 μequiv/L; De la Trinité, pH 5.2, alkalinity 16 μequiv/L. Before and after the spring flood, all pH values lay in the circumneutral range.

These episodes of decreased pH and alkalinity were characterized by sustained dilution of the base cations (e.g. 35–55% decrease in [Ca] from peak values observed just before the spring thaw). The strong acid anions (SO_4, NO_3) were also subject to dilution during the period of maximum discharge.

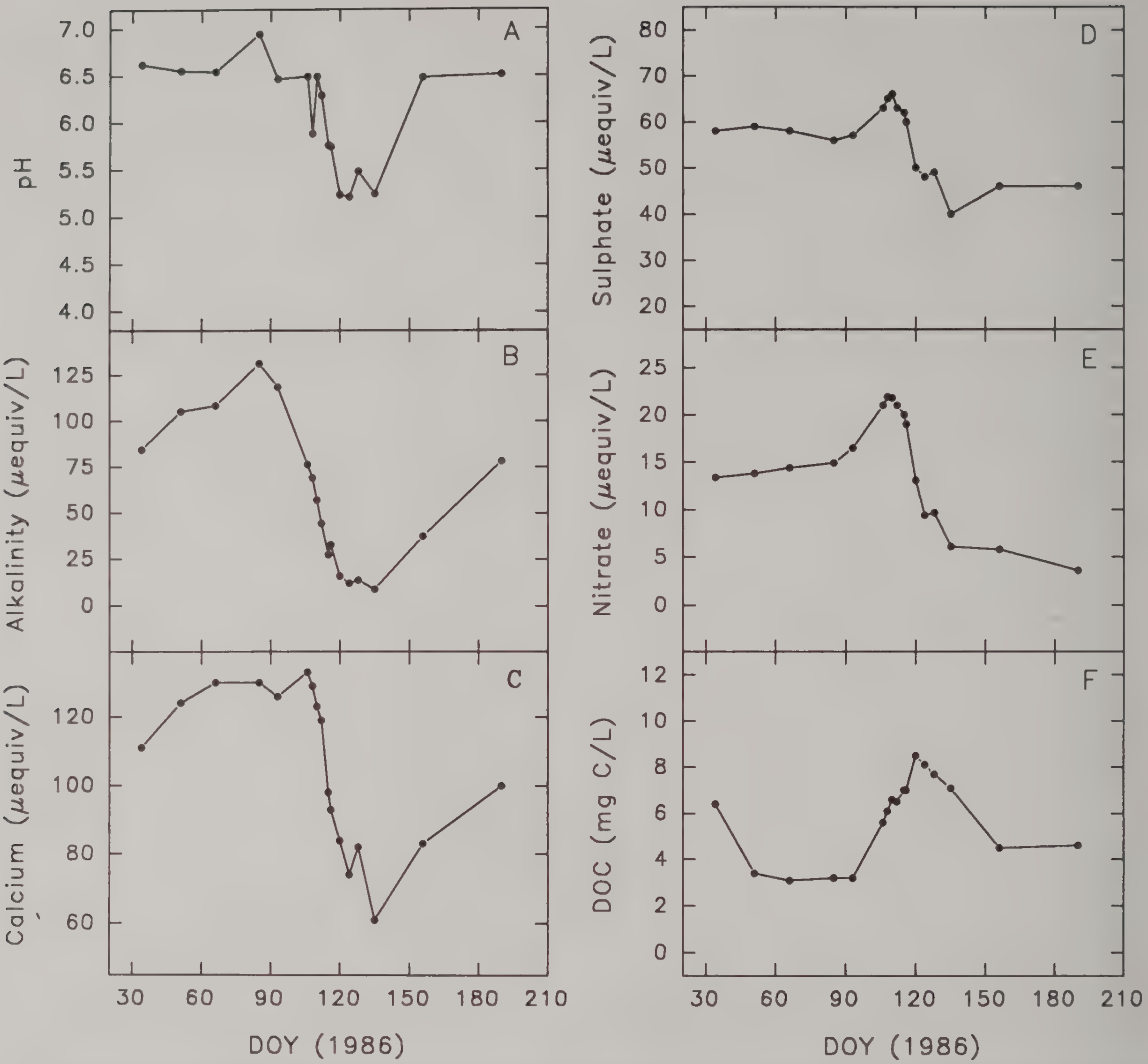

FIG. 3. Water chemistry changes during spring runoff at the De la Trinité River site (1986). (A) pH; (B) Gran alkalinity; (C) Ca; (D) SO_4; (E) NO_3; (F) DOC.

However, at the sampling site on the De la Trinité River (Fig. 3D, 3E), an apparent pulse of SO_4 and NO_3 could be observed very early in the spring runoff period, followed by a decline in concentrations to values less than those observed during the winter months. A similar response was noted for SO_4 (but not NO_3) at the Aux Rochers site (Fig. 4D, 4E). These short-term concentration maxima occurred from day 105 to 115, i.e. during the very first stages of snowmelt, when river discharge had only just begun to respond (compare Fig. 2, days 105–115). Note that there was no evidence of these early pulses at the site on the McDonald River (Fig. 5D, 5E).

In distinct contrast with the general patterns of dilution observed for the other solutes, marked *increases* in DOC (100–150%) and water colour (50–130%) occurred during spring runoff, with peak values being observed on approximately day 120 (Fig. 3F, 4F, 5F). Ion balance calculations indicate that the anionic deficit (Σ cations − Σ anions) also attained maximum values during this period (De la Trinité, 70 μequiv/L; Aux Rochers, 100 μequiv/L; McDonald, 90 μequiv/L); anion deficits before snowmelt averaged only ≈35 μequiv/L.

Al Geochemistry during Snowmelt

Moderate concentrations of "dissolved" Al (5–6 μM $[Al]_d$) were observed at the beginning of the study period at all three sites, presumably reflecting the slight thaw that had occurred just prior to the first sampling; concentrations declined slowly during the ensuing period of diminishing discharge. The speciation of this dissolved Al, i.e. its distribution among the various operationally defined forms, was relatively constant during this premelt period. The results for the De la Trinité River (Fig. 6) are typical of those observed at the other two sites: February to mid-April: monomeric inorganic Al ≈15%, monomeric organic Al ≈35%, and non-PCV-reactive Al ≈ 50%.

The subsequent period of spring runoff was characterized by increases in the dissolved Al concentrations (peak values of 6–

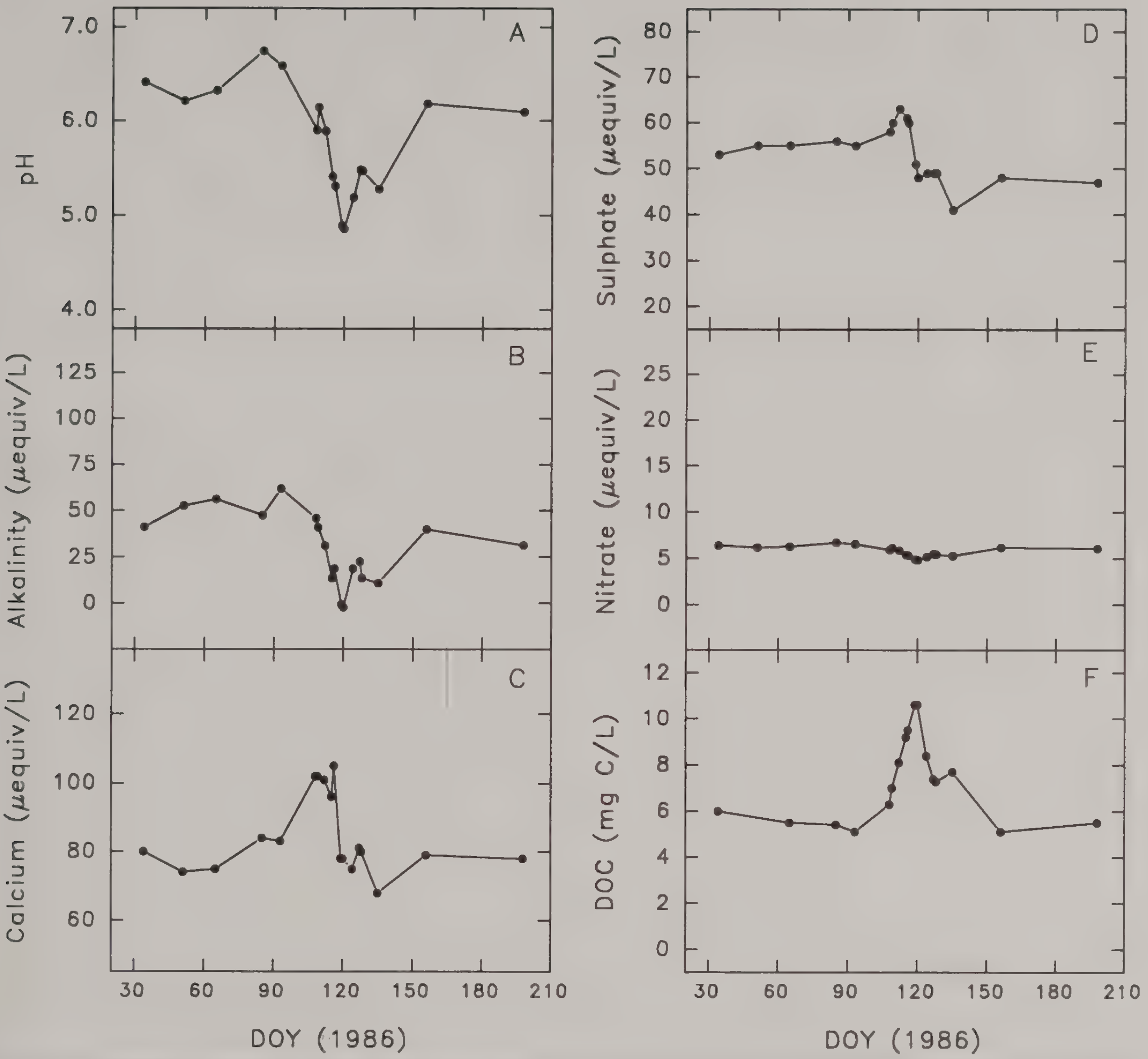

FIG. 4. Water chemistry changes during spring runoff at the Aux Rochers River site (1986). (A) pH; (B) Gran alkalinity; (C) Ca; (D) SO_4; (E) NO_3; (F) DOC.

8 µM $[Al]_d$ in the three rivers) and by changes in its speciation. Both monomeric forms of Al contributed to the spring Al peak whereas the non-PCV-reactive forms of Al (e.g. polynuclear species, hydroxy–Al colloids, nonreactive forms associated with DOC) declined in importance (20–30%). Concentrations of monomeric *inorganic* Al increased about threefold (<0.8 to 2.3–2.4 µM) in all three rivers during the first phase of spring runoff; levels decreased somewhat after this first flush and then remained relatively constant (≈1.2 µM) during the second runoff phase. Monomeric *organic* Al also increased noticeably during the snowmelt period, with similar average concentrations being observed in the three rivers (2.7–2.9 µM).

The distribution of Al among its various inorganic complexes, as calculated with the ALCHEMI chemical equilibrium model, also showed marked fluctuations during the snowmelt period (e.g. Fig. 7). Mixed ligand $AlOH(F)_n$ complexes, together with the cationic hydroxy–Al complexes ($AlOH^{2+}$ and $Al(OH)_2{}^+$), dominated the calculated speciation of dissolved inorganic Al for all three rivers during the winter low flows. With the advent of spring runoff, however, the pH depression led to a marked reduction in the relative proportions of these species; the fluoro–Al complexes (AlF^{2+} and $AlF_2{}^+$), together with free Al^{3+}, assumed much greater importance. Even during this period of low pH, however, the calculated proportion of free Al^{3+} never exceeded 40%. As the pH rose after the spring flood, the hydroxy–Al complexes and the mixed ligand $AlOHF_n$ species resumed their former dominance.

Seasonal Variability in Al–DOC Interactions

Water samples were collected from the De la Trinité River before, during, and after spring runoff in 1988. The hydrophobic fulvic and humic acid fractions of the DOM, as isolated by adsorption from the acidified water samples onto an XAD-8 resin, represented about 50% of the DOC originally present. When these fractions were titrated with Al, the concentration

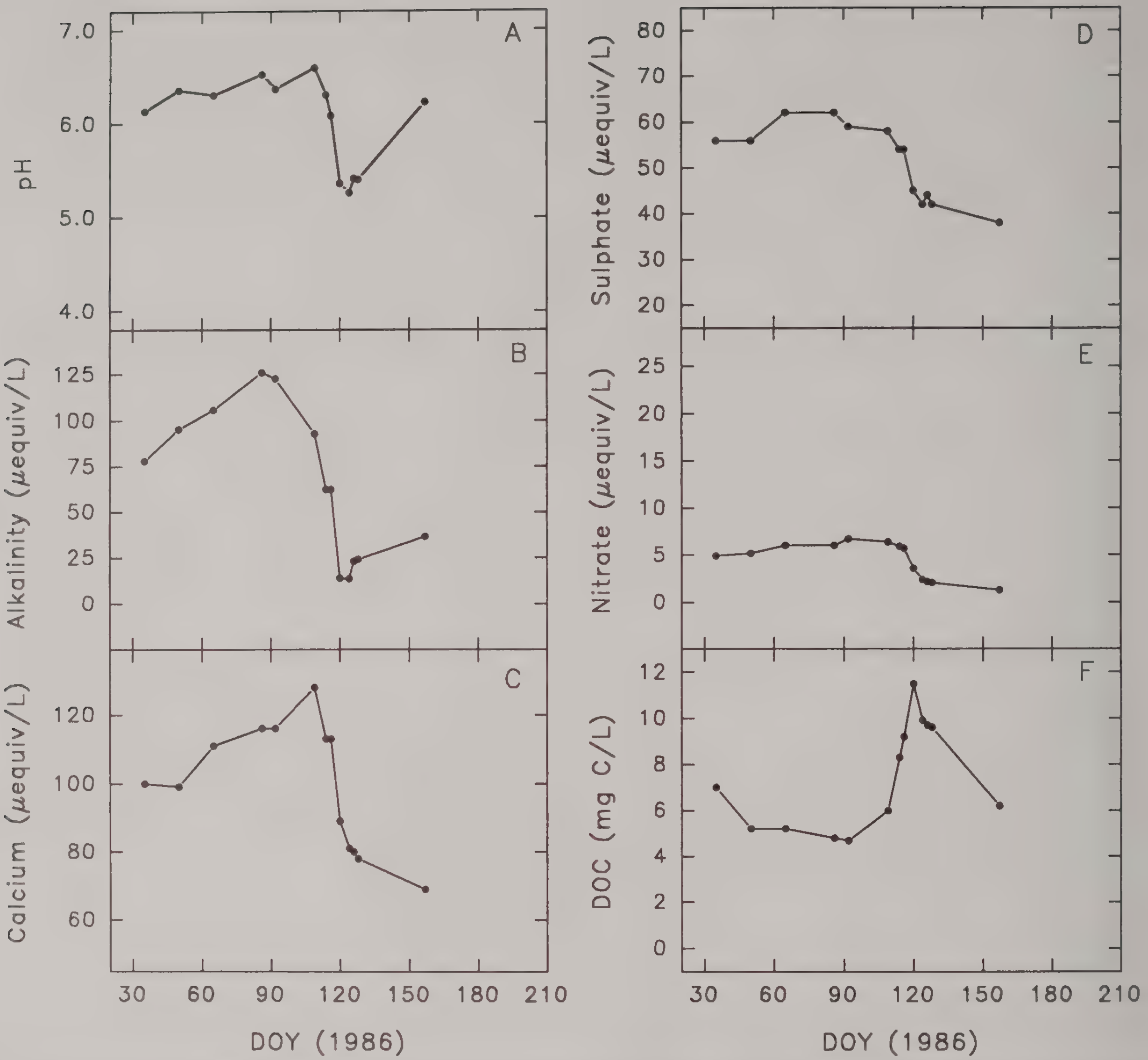

FIG. 5. Water chemistry changes during spring runoff at the McDonald River site (1986). (A) pH; (B) Gran alkalinity; (C) Ca; (D) SO_4; (E) NO_3; (F) DOC.

of bound Al increased progressively, tending towards a plateau. Data analysis was confined to the area of the titration curve where $[Al]_{bound}$ was still sensitive to changes in $[Al]_{added}$, i.e. up to but not beyond saturation of the complexation capacity. When examined by Langmuir regression analysis ($[Al]_{bound}/[Al]_{free}$ versus $[Al]_{free}$; Pott et al. 1985), each titration curve yielded a reasonable straight line ($r^2 = 0.95$–0.99) from which could be extracted an overall conditional stability constant (K_c) and an estimate of the complexation capacity (CC, micromoles Al per milligram C) (Table 1).

Discussion

Episodic Acidification

Episodic acidification, defined as a short-term depression in pH or alkalinity associated with an increase in discharge, is a well-documented phenomenon in running waters. Such episodes were first associated with snowmelt (e.g. Johannessen et al. 1980) but have since been shown also to occur during summer and autumn spates (for a recent review, see Wigington et al. (1990)). Based on the present results, the De la Trinité, Aux Rochers, and McDonald rivers all experience episodic acidification during the spring runoff period; earlier suggestions in the literature, based on monthly sampling of these and other North Shore salmon rivers (Walsh et al. 1987; Lachance et al. 1988), are thus confirmed.

Causes

During acidification episodes, decreases in pH and loss of alkalinity are usually accompanied by dilution of the base cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and/or by an increase in the concentrations of the strong acid anions (SO_4^{2-}, NO_3^-) (Molot et al. 1989; Wigington et al. 1990); less frequently, increases in organic acid anions have been reported. In the present case, base cation dilution is indeed observed (Fig. 3C, 4C, 5C),

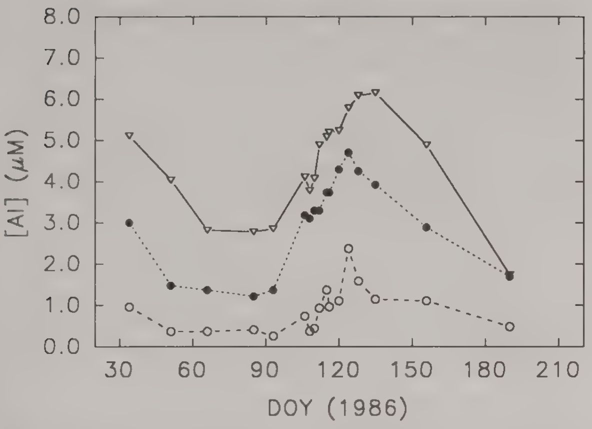

FIG. 6. Changes in Al speciation during spring runoff at the De la Trinité River site (1986). Open circles = $[Al]_{pcv}$ − $[Al]_{nex}$; solid circles = $[Al]_{pcv}$; open triangles = $[Al]_d$. See Materials and Methods section for details of the analytical methodology.

accompanied by dilution of the acid anions and marked increases in DOC (Fig. 3F, 4F, 5F).

To evaluate the relative importance of mineral and organic acids to the pH depression, the organic acid anions, $RCOO^-$, were estimated by charge balance (Cronan et al. 1978). The concentrations of SO_4^{2-}, NO_3^-, and $RCOO^-$ were then normalized with respect to the sum of the base cations, to take dilution into account, and these normalized values were plotted over time (Fig. 8A, 8B, 8C). From these figures, it is clear that organic acids play a key role during the spring pH and alkalinity depression at all three sites. At the site on the De la Trinité River, the normalized concentrations of SO_4^{2-} and NO_3^- do increase slightly, early in the melt period (Fig. 8A, days 115–125), suggesting that strong mineral acids may contribute to the early phase of the acidification episode in this river. Such early pulses of acid anions have been reported in several other snowmelt studies (e.g. Molot et al. 1989; Schaefer et al. 1990). However, as can be seen from a calculation of the relative contributions of the various anions at the early pH minimum observed in each river, even at the De la Trinité site the organic anion contribution exceeded the combined contributions of SO_4^{2-} and NO_3^- (microequivalents per litre).

River	Date	pH	NO_3^-	SO_4^{2-}	$RCOO^-$
De la Trinité	3 May	5.2	9	48	64
Aux Rochers	29 April	4.9	3	48	98
McDonald	3 May	5.3	2	42	87

The relative contributions of base cations, mineral acid anions, and organic acid anions to decreases in alkalinity during snowmelt were also calculated according to the charge-balance approach employed by Molot et al. (1989). Changes in alkalinity, $[SO_4^{2-}]$, $[NO_3^-]$, $[RCOO^-]$, and C_b ($Ca^{2+} + Mg^{2+} + Na^+ + K^+$) were calculated as the difference between the daily value during the episode and a pre-episode value (average value from the period of low flow that preceded snowmelt). Daily ion-specific contributions to alkalinity increases and decreases during snowmelt (Df_i) were then calculated as $\delta SO_4/\delta alk$, $\delta NO_3/\delta alk$, $\delta Cl/\delta alk$, $\delta C_b/\delta alk$, and $\delta RCOO^-/\delta alk$ where

δalk = alkalinity (pre-event) − alkalinity (day i)
$\delta C_b = C_b$ (pre-event) − C_b (day i)
$\delta SO_4 = SO_4^{2-}$ (day i) − SO_4^{2-} (pre-event)
$\delta NO_3 = NO_3^-$ (day i) − NO_3^- (pre-event)
$\delta Cl = Cl^-$ (day i) − Cl^- (pre-event)
$\delta RCOO^- = RCOO^-$ (day i) − $RCOO^-$ (pre-event)

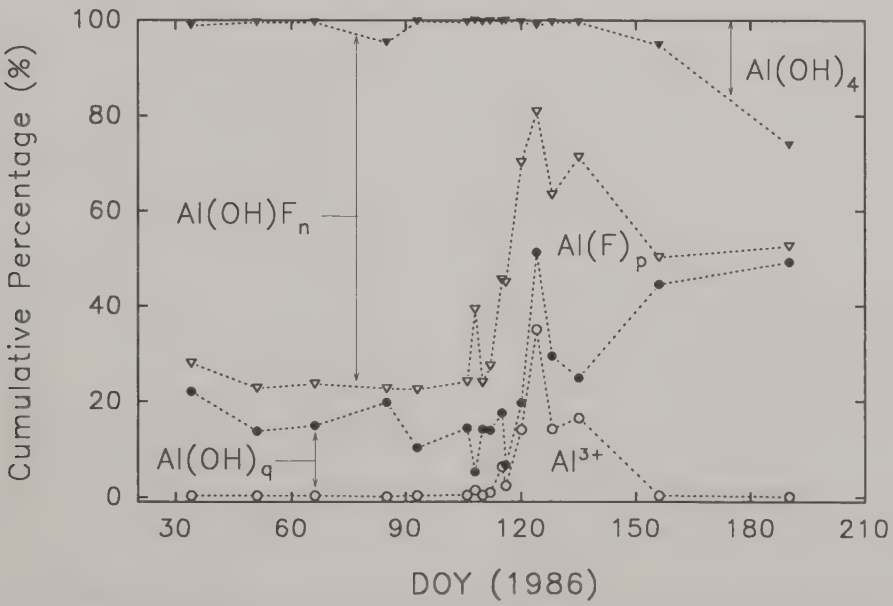

FIG. 7. Changes in the speciation of dissolved inorganic Al during spring runoff at the De la Trinité River site (1986). Distribution of monomeric Al among its various inorganic complexes as calculated with ALCHEMI, a chemical equilibrium model. For a given sampling date, the cumulative percentages are calculated in the following order: Al^{3+}, $Al(OH)_q$, AlF_p, $Al(OH)F_n$, and $Al(OH)_4^-$.

TABLE 1. Complexation parameters for the titration of isolated fulvic and humic acid fractions with inorganic Al (De la Trinité River, 1988).

Sample	Date	Langmuir complexation parameters[a]		
		log K	CC (μmol Al/mg C)	r^2
Fulvic acid	31 March	6.17	1.01 ± 0.03[b]	0.99
	5 May	6.31	0.74 ± 0.02	0.99
	24 August	6.23	0.72 ± 0.06	0.95
Humic acid	31 March	6.05	0.59 ± 0.02	0.98
	5 May	6.01	0.80 ± 0.04	0.98
	24 August	6.04	0.82 ± 0.02	0.99

[a] Complexation parameters obtained by regression analysis, $[\mathrm{Al}]_{\mathrm{bound}}/[\mathrm{Al}]_{\mathrm{free}} = 1/(K\cdot\mathrm{CC}\cdot[\mathrm{DOC}]) + [\mathrm{Al}]_{\mathrm{free}}/(\mathrm{CC}\cdot[\mathrm{DOC}])$; slope $= 1/(\mathrm{CC}\cdot[\mathrm{DOC}])$, y-intercept $= 1/(K\cdot\mathrm{CC}\cdot[\mathrm{DOC}])$.

[b] We have estimated the uncertainty of the CC values from the standard error of the slope, assuming that [DOC] had been measured without error.

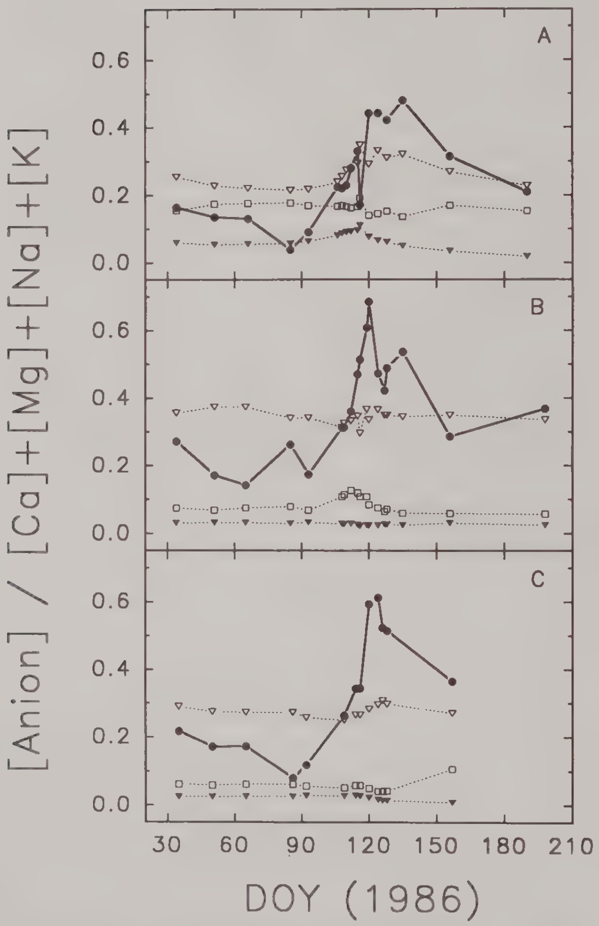

FIG. 8. Changes in the normalized concentrations of SO_4^{2-} (open triangles), NO_3^- (solid triangles), Cl^- (open squares), and $RCOO^-$ (solid circles) during spring runoff (1986). Normalized concentration = [anion]/([Ca] + [Mg] + [Na] + [K]). (A) De la Trinité River; (B) Aux Rochers River; (C) McDonald River.

and all concentrations are expressed as microequivalents per litre. Positive values of df_i denote contributions to alkalinity decreases and negative values represent contributions to alkalinity increases. The theoretical sum of the five contributions on any given day (Σdf_i) is 1.0 (Molot et al. 1989).

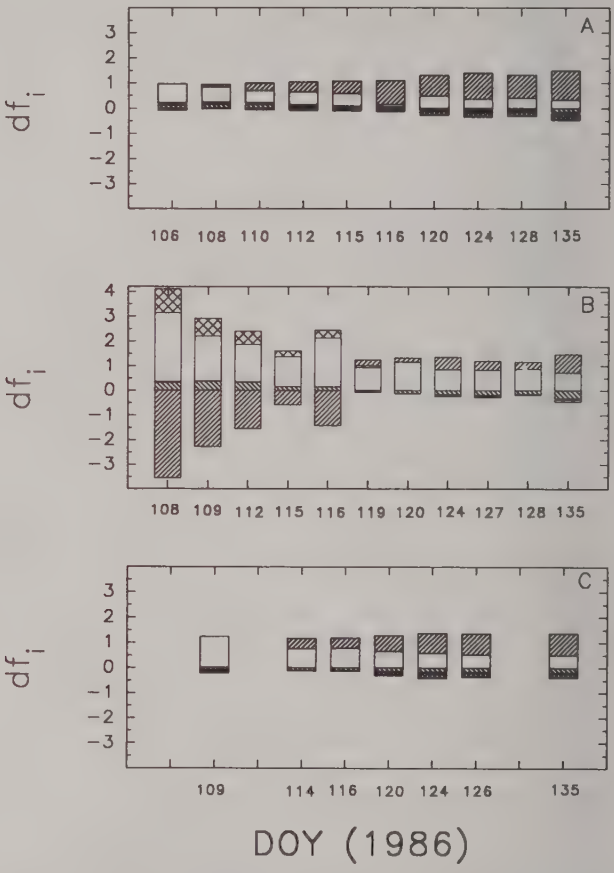

FIG. 9. Relative contributions of changes in C_b (left hatch), SO_4^{2-} (right hatch), NO_3^- (solid), Cl^- (cross-hatch), and $RCOO^-$ (open) to alkalinity depressions (df_i). Contributions are expressed as δion/δalk where δ is the difference between a pre-episode value and the concentration on a given day. (A) De la Trinité River; (B) Aux Rochers River; (C) McDonald River.

The average Σdf_i values for the three rivers are indeed close to unity (De la Trinité, 0.96 ± 0.05, n = 10; Aux Rochers, 0.95 ± 0.20, n = 11; McDonald, 0.99 ± 0.03, n = 7), suggesting that the analysis is valid (in a similar analysis of data from the outlets of a number of lakes in the Adirondack mountains, New York, Schaefer et al. (1990) found that only 7 of 21 Σdf_i values fell within 20% of the theoretical value of

1.0; note, however, that they did not consider the possible contribution of organic acids). Increases in $RCOO^-$ dominate the alkalinity depression episodes (i.e. $\delta RCOO^-/\delta alk$ consistently affords the largest positive df_i numbers; see Fig. 9). For the McDonald and De la Trinité rivers, this dominance is most marked during the rising limb of the snowmelt hydrograph (days 105–115); during peak discharge and the recession phase, the contribution of base cation dilution becomes more obvious (Fig. 9A, 9C). Schaefer et al. (1990) reported a similar trend in their study of Adirondack lakes, where the latter phases of springtime alkalinity depression also tended to be dominated by C_b dilution. In the case of the Aux Rochers River, the dominance of the organic anions persists to the end of the episode; base cation concentrations actually increase during the early runoff period (note the negative $\delta C_b/\delta alk$ values in Fig. 9B). The contributions of the inorganic acid anions (SO_4^{2-}, NO_3^-, Cl^-) to alkalinity depression always rank below those of $\delta RCOO^-$ and δC_b; indeed, only in the case of the Aux Rochers River do these contributions appear to play a significant role (note the contribution of δSO_4 and δCl for days 108–112 in Fig. 9B).

Clearly both approaches (i.e. normalized [anion]/C_b plots or successive df_i calculations) indicate that organic acids play a key role in the episodic acidification of the three North Shore rivers.

Comparison with the episodic acidification literature

Our understanding (indeed, even the definition) of episodic acidification has evolved markedly over the last 5–10 yr. Initially such episodes were widely thought to result from the direct input into streams of acidic water of atmospheric origin (snowmelt or rain events), with little contact having occurred between this dilute rain or meltwater and the soil exchange compartment (Johannessen et al. 1980); the influence of anthropogenic acid deposition was stressed. It is now realized that "pre-event" water contributes very significantly to snowmelt/storm hydrographs (Christophersen and Neal 1990; Wels et al. 1990) and that natural phenomena (C_b dilution, nitrification, organic acid leaching) may contribute to episodic losses of alkalinity.

Despite this evolution in thinking, however, and despite the recognized potential of natural organic acids to contribute to the *chronic* acidity of surface waters (Gorham et al. 1986; Kerekes et al. 1986; Dupont 1991; Wilkinson et al. 1992), organic acids have consistently been reported to play a negligible role in *episodic* acidification (Wigington et al. 1990). Virtually all reports of episodic acidification in the literature have stressed dilution of base cations and increased relative concentrations of either SO_4^{2-} (e.g. Christopherson et al. 1984; LaZerte and Dillon 1984; Clair and Freedman 1986; Kerekes et al. 1986; Molot et al. 1989) or NO_3^- (e.g. Driscoll et al. 1987; Schaefer et al. 1990). In the former case (SO_4^{2-}), anthropogenic acid deposition has been implicated as the source of acidity (LaZerte and Dillon 1984) whereas for NO_3^-, it has proven understandably difficult to evaluate the relative importance of nitrification in the forest litter layer versus atmospheric deposition of NO_3^- (Wigington et al. 1990).

A survey of the recent literature did turn up a number of reports where decreases in pH or alkalinity had been accompanied by rises in DOC (e.g. Edwards et al. 1986; Lacroix and Kan 1986; Goenaga and Williams 1988), but since changes in base cations, SO_4^{2-}, and NO_3^- were generally not reported, it proved difficult if not impossible to evaluate the relative importance of organic acid anions in these cases. Two of the more complete studies are compared with our results in Table 2.

Marked differences exist between the various catchments represented in Table 2 and the Quebec North Shore rivers. For example, the earlier studies were all carried out in small (research) watersheds (<3 km^2) on low-order, often headwater streams that were chronically acidic (see pH^i column). In contrast, our study has focused on three large, high-order rivers; although dilute and relatively unbuffered, they are circumneutral throughout most of the year. It should also be recalled that the North Shore catchments are remote, situated well downwind from major sources of airborne pollutants, and thus receive lower atmospheric loading of strong mineral acids than do the other catchments listed in Table 2.

To judge from Table 2, such previous evidence as does exist for the contribution of organic acids to episodic acidification is limited to storm events occurring in low-order streams during the summer or autumn months. Indeed, in the recent review document prepared for the U.S. National Acid Precipitation Assessment Program (Wigington et al. 1990), it is suggested that "organic acids are unlikely to be important contributors to the acidity of episodes during snowmelt or in high order streams." *Our results clearly demonstrate that these generalizations cannot be extended to the Quebec North Shore.*

Despite the absence of detailed information on soil and groundwater chemistry in the catchments of the three studied rivers, it is tempting to speculate that the important contribution of organic acids to the spring pH and alkalinity depression reflects the routing of the meltwater through the upper organic soil horizons. Note in this connection that coniferous vegetation produces higher soil water DOC concentrations than does deciduous vegetation (Cronan and Aiken 1985). Data available for ferro-humic and humo-ferric podzols elsewhere on the Precambrian Shield (e.g. Hendershot et al. 1984) indicate that the organic horizon is an important source of DOC and acidity; normally this DOC is efficiently retained in the lower mineral soils, by precipitation or adsorption, but when flowpaths bypass the mineral soil the forest soil can export organic acids. Cation dilution is also likely to occur under such conditions (Wigington et al. 1990).

Wetlands are also present in these catchments (Ducruc 1985; Environment Canada 1985). Were snowmelt to result in a piston-flow contribution of high DOC water from such off-channel sources, increases in riverwater DOC concentrations might be expected. However, quantitative data that would allow estimation of the relative importance of these two sources of DOC (wetlands versus organic soil horizons) are not available at the catchment scale.

Al Geochemistry during Spring Snowmelt

Having considered in some detail the episodic acidification of the Quebec North Shore salmon rivers, we now turn our attention to the geochemical behaviour of Al during the same snowmelt period. Controls on the solubility of Al and changes in its speciation, notably the distribution between inorganic and organic forms, are emphasized.

Solubility control

Since the first reports that Al concentrations in surface waters increase in response to environmental acidification, many researchers have considered the question of solubility control. Early studies, mostly on standing water bodies, indicated that Al concentrations could be reasonably predicted on the basis of the precipitation–dissolution reactions of an Al trihydroxide phase, $Al(OH)_3(s)$, the solubility of which was close to that of microcrystalline gibbsite ($K_{so} = 10^{-9.35}$) (e.g. Campbell et al.

TABLE 2. Documented examples of episodic acidification attributed to inputs of organic acids.[a]

Study site	Month	Area	pH^i	δH^+	C_b^i	C_b^e	Alk^i	Alk^e	DOC^i	DOC^e	Def^i	Def^e	SO_4^i	SO_4^e	NO_3^i	NO_3^e
Svartbergret, Sweden[b]	July	0.5	5.5	53	205	240 185	—	—	12	25	—	—	90	120 <80	<10	<10
Bickford Reservoir, Massachusetts[c]	November	2.2	5.3	17	285	345 295	−4	−27	5	11	—	—	240[d]	290 250	—[d]	—[d]
Present study																
De la Trinité	April–May	562	6.5	59	261	125	125	9	3.2	8.5	23	76	58	66 40	16	22
Aux Rochers	April–May	4170	6.5	135	152	119	55	−2	5.3	10.6	32	104	55	63 41	5	3
McDonald	April–May	682	6.4	51	228	141	124	14	5.0	11.5	23	94	61	42	6	2

[a] Explanation of symbols: δH^+ = change in H^+ concentration during event; C_b = base cations; Alk = alkalinity; DOC = dissolved organic carbon; Def = anion deficit = Σcations − Σanions. Superscript i = initial conditions; superscript e = conditions during the event. Where two values are given for the event, the first corresponds to a transient peak early in the episode and the second to the subsequent minimum. All units are in µequiv/L, except for DOC (mg C/L) and watershed area (km^2).

[b] Svartbergret research catchment, weir "B" (Bishop et al. 1990).

[c] Bickford Reservoir wetland tributary (McAvoy 1989).

[d] Results reported as the sum of SO_4^{2-} and NO_3^-.

1984; Driscoll et al. 1984; LaZerte 1984). However, several more recent studies have revealed cases where the observed Al concentrations are well below those predicted by this relationship (e.g. Cronan et al. 1986) and indeed events where [Al] has failed to increase in response to a decrease in pH (e.g. Sullivan et al. 1986).

To check for seasonal changes in solubility control, saturation indices (SI) were calculated for each river with respect to microcrystalline gibbsite (SI = log ($\{Al^{3+}\}\cdot\{OH^-\}^3/K_{so}$)); SI values >0 indicate oversaturation. Marked seasonal fluctuations are indeed observed, with a similar pattern occurring for each river. For the De la Trinité and Aux Rochers rivers (Fig. 10A, 10B), values are close to or above the saturation limit for samples collected before or after the snowmelt period, but during maximum discharge, SI drops well below the horizontal line that corresponds to microcrystalline gibbsite. A similar seasonal trend is also observed for the McDonald River (Fig. 10C), although the SI values fluctuate somewhat less than those calculated for the other two sites.

In surface waters, such cases of undersaturation are usually encountered in two situations (Cronan et al. 1986): at sites strongly influenced by wetland drainage or in samples collected during periods of high discharge. The observed undersaturation has been attributed either to kinetic constraints (i.e. during high-flow events the dissolution of $Al(OH)_3(s)$ occurs too slowly to maintain the system in equilibrium (Hooper and Shoemaker 1985; Sullivan et al. 1986)) or to the adsorption or ion-exchange of Al onto solid humic material in the soil matrix, a reaction that would maintain [Al^{3+}] at levels lower than those that would normally exist in equilibrium with $Al(OH)_3(s)$ (Cronan et al. 1986; Walker et al. 1990). This latter explanation clearly is consistent with the changes in flowpath during snowmelt that were invoked earlier to explain the contribution of organic acids to the spring acidification episode.

Al speciation

Given the known affinity of Al for fulvic acids, and the documented sensitivity of Al speciation to pH changes in experimental systems (Campbell and Tessier 1987; Tipping and Backes 1988), one might expect Al speciation in natural waters to be influenced by variations in DOC and pH. Recent studies of the kinetics of Al complexation reactions (e.g. Plankey and Patterson 1987, 1988) suggest that Al speciation should indeed respond rapidly to changes in H^+ and DOC concentrations and

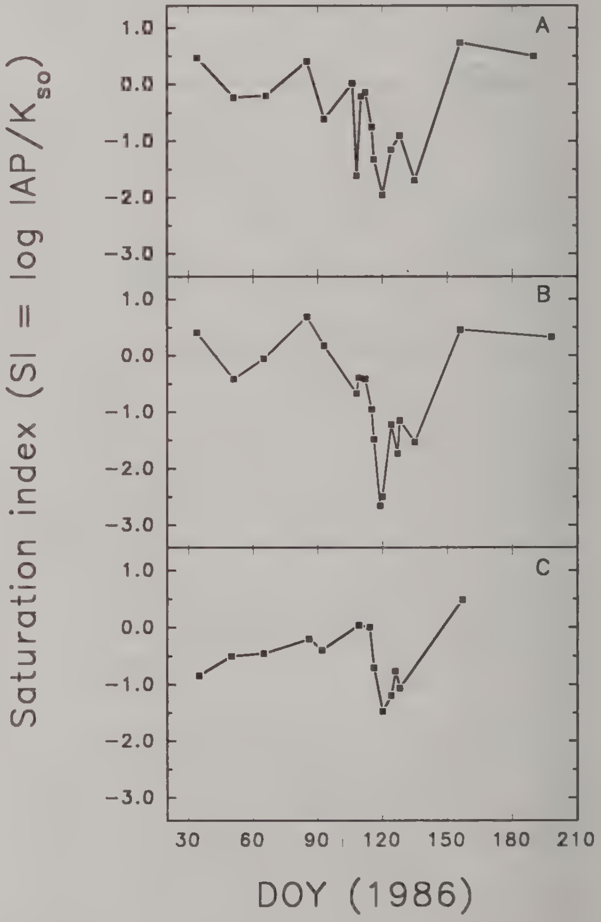

FIG. 10. Changes in saturation indices (SI) with respect to microcrystalline gibbsite (SI = log ($\{Al^{3+}\}\cdot\{OH^-\}^3/K_{so}$)) during spring runoff (1986). SI values >0 indicate oversaturation. (A) De la Trinité River; (B) Aux Rochers River; (C) McDonald River.

that the prediction of Al speciation in such systems should be amenable to an equilibrium approach (Campbell et al. 1986). The present data, covering a wide range of DOC (3–12 mg C/L) and H^+ concentrations (0.1–40 μequiv/L), offer a challenging case for testing this approach.

As a first approach, we adopted the empirical equilibrium model that Backes and Tipping (1987) had developed for an isolated DOM fraction (i.e. their Model I). This model is based on a linear logarithmic relationship

$$(1)\quad \log_{10}\vartheta = \log_{10}\alpha + \beta\cdot\log_{10}\{Al^{3+}\} + \tau\cdot\log_{10}\{H^+\}$$

which can also be expressed as

$$(2)\quad \vartheta = \alpha\cdot\{Al^{3+}\}^{\beta}\cdot\{H^+\}^{\tau}$$

where ϑ = moles of Al bound per gram of humic substance (HS) and α, β, and τ are empirical constants. It follows that concentrations of organic bound Al, [Al-org], can be calculated as

$$(3)\quad [\text{Al-org}] = \alpha\cdot\{Al^{3+}\}^{\beta}\cdot\{H^+\}^{\tau}\cdot[\text{HS}].$$

As can be seen by inspection of equation 2, β and τ account for the (competitive) interactions of H^+ and Al^{3+} with HS whereas α is a capacity factor.

In applying their Model I to a variety of natural waters, Tipping et al. (1988) first assumed that [HS] = 2 × [DOC], i.e. that the measured DOC was entirely composed of HS with a C content of 50%, and then held α, β, and τ constant at values previously determined in the laboratory for an isolated, high molecular weight DOM fraction ($\alpha = 1.32 \times 10^{-4}$, $\beta = 0.718$, $\tau = -1.054$). Although this approach yielded quite reasonable agreement between measured and calculated values of [Al-org] ($r = 0.93$), Tipping et al. (1988) obtained a somewhat better fit by "rescaling" α, the capacity factor. They performed this rescaling for each individual set of field observations and for the combined data set; in the latter case the optimal agreement between measured and calculated values was obtained with $\alpha = 1.84 \times 10^{-4}$. Note that fixing β and τ, while allowing α to vary, amounts to assuming that all HS have similar affinities for H^+ and for Al^{3+} but that different HS may have different binding capacities.

We adopted a similar approach for the Quebec North Shore salmon rivers. The Backes and Tipping model was initially employed with the original laboratory-derived constants, but for each river the calculated values of [Al-org] greatly exceeded the field values, particularly during the snowmelt period (predicted values were up to 14× measured values); typical results are shown in Fig. 11A for the De la Trinité River. From even a cursory inspection of this plot, it is clear that the failure of the Backes and Tipping model cannot be remedied by simply "rescaling" α; predicted and measured values of [Al-org] are only correlated very weakly if at all (De la Trinité, $n = 17$, $r = 0.46$; Aux Rochers, $n = 17$, $r = 0.04$, NS; McDonald, $n = 13$, $r = 0.45$; all three rivers, $n = 47$, $r = 0.32$). It should be noted in this context that we are perhaps asking the original model "to perform too well" (E. Tipping, Institute of Freshwater Ecology, Windermere Laboratory, U.K., pers. comm.).

A reasonably satisfactory agreement between measured and calculated values of [Al-org] was only obtained by allowing α, β, and τ to float (cf. LaZerte 1989). Multiple linear regression on the pooled data set for all three rivers *outside the snowmelt period* yielded the following equation:

$$(4)\quad [\text{Al-org}] = \alpha\cdot\{Al^{3+}\}^{\beta}\cdot\{H^+\}^{\tau}\cdot[\text{DOC}]^{\Theta}$$

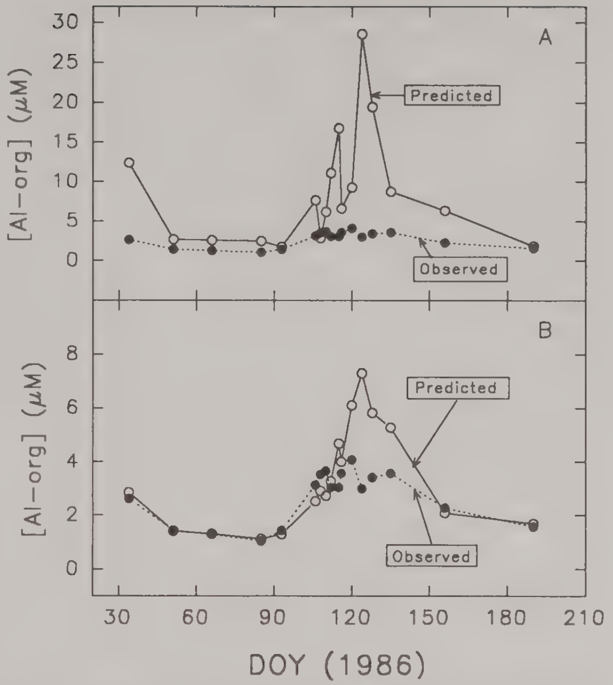

FIG. 11. Predicted (open circles) and observed (solid circles) concentrations of monomeric organic Al during spring runoff at the De la Trinité River site (1986). Predicted values were calculated with a linear logarithmic model using (A) the original Backes and Tipping (1987) constants and (B) revised constants derived from multiple regression analysis (see text for details).

with $\alpha = 3.55 \times 10^{-3}$, $\beta = 0.129$, $\tau = 0$, and $\Theta = 0.91$). There are several important differences between this result and the original Backes and Tipping model: β, the exponent for $\{Al^{3+}\}$, is much smaller than the original constant; τ, the exponent for $\{H^+\}$, has dropped to 0, indicating that the pH has effectively disappeared from the predictive equation; Θ, assumed to be equal to 1.0 in the Backes and Tipping model, has decreased to 0.91. Very similar results were obtained by LaZerte (1989) when he attempted to apply the Backes and Tipping model to Al speciation data from surface waters in central Ontario. He too found that pH contributed little to the regression; the high correlation among pH, $\log_{10}$[DOC], and $\log_{10}[Al^{3+}]$ effectively made pH a redundant variable. Note that the disappearance of pH from the predictive equation does NOT imply that variations in pH do not affect Al–DOM interactions, but rather that pH changes can induce opposing or compensating effects. For example, decreases in pH will normally lead to higher $\{Al^{3+}\}$, favouring complexation; simultaneously, the higher $\{H^+\}$ will promote increased protonation of HS functional groups, hindering complexation. In addition, in a field setting, lower pH values may be linked to increased [HS], favouring complexation. In the present case, these various effects seem to offset each other, and accordingly, [Al-org] appears to be insensitive to pH variations.

The improved fit afforded by the modified Backes and Tipping equation can be appreciated by comparing Fig. 11A and 11B (note the change in scale); agreement between predicted and measured values for [Al-org] is clearly much better with the adjusted constants. Close inspection of Fig. 11B (and the corresponding plots for the other two rivers) shows that the modified Backes and Tipping model performs well *outside* the

TABLE 3. Toxicity of Al and H^+ to juvenile Atlantic salmon. Compilation of bioassay results from the literature and comparison with conditions in the North Shore salmon rivers during snowmelt.

Type of study	[Al] (μM)[a]	pH	[Ca] (μM)	Biological response	Reference
Field observations	1.5	5.1	20	Smolt mortality	Henriksen et al. 1984
Field observations	1.9–3.0	5.0	20	Smolt mortality	Skogheim et al. 1986
Field bioassay (smolt 2+)	2.3	4.6	15	100% mortality (6d)	Rosseland and Skogheim 1984
Field bioassay (smolt 1+)	2.2±0.3	4.6	15	100% mortality (2.5 d)	Rosseland et al. 1986
Field bioassay (smolt 2+)	2.5±0.8	4.8	33	100% mortality (6 d)	Rosseland and Skogheim 1986
Field bioassay (smolt 2+)	1.4±0.3	5.1	33	0% mortality (2.5 d)	Skogheim et al. 1986
Field bioassay (added Al, smolt 2+)	1.4 2.8 5.1	5.1	33	0% mortality 100% mortality[b] 100% mortality[b]	Skogheim and Rosseland 1986
De la Trinité	1.4±0.5	5.2–5.8	41±6	—	Present study
Aux Rochers	1.5±0.5	4.9–5.5	42±6	—	
McDonald	1.8±0.5	5.3–5.4	41±3	—	

[a] Concentrations of labile inorganic Al (ΣAl^{3+} + monomeric inorganic complexes).

[b] Median lethal time 108 h at 2.8 μM Al, 38 h at 5.1 μM Al.

snowmelt period (e.g. $n = 19$, $r^2 = 0.92$ for samples collected from the three rivers before or after spring runoff), but its performance deteriorates during the period of maximum discharge. In effect, concentrations of Al-org predicted for the key spring runoff period, although more reasonable than those calculated from the original Backes and Tipping equation, nevertheless consistently exceed measured concentrations. This positive bias is somewhat greater for the McDonald River (4–6 μM) than at the other two sites (Aux Rochers River, 3–4 μM; De la Trinité River, 2–3 μM). The initial failure of the Backes and Tipping model, most obvious during snowmelt, and the persistent overestimation of Al-org during this period, even after model recalibration, led us to test for seasonal variations in the Al-complexing properties of river water DOM (specifically a weakening of the Al–DOM interaction during spring runoff).

Seasonal variability in Al–DOM interactions

Hydrophobic fulvic and humic acids, isolated from water samples collected from the De la Trinité River before, during, and after spring runoff in 1988, were titrated with Al. The measured complexation parameters (Table 1) are similar to those reported in the literature (Pott et al. 1985) and are remarkably constant over the sampling period, showing no seasonal trend. In particular, there is no evidence that the fulvic/humic acid fractions isolated during the period of peak discharge (May 1988) bind Al more weakly than the material isolated before or after snowmelt. We conclude that the Al-complexing properties of the hydrophobic fulvic/humic acid component of river water DOM do not vary appreciably during spring runoff; the consistent overestimation of [Al-org] during this period presumably reflects the presence in the spring DOM peak of an appreciable nonhumic component (hydrophilic, with different Al-binding characteristics). Consistent with this suggestion is the observation that the ratio (water colour/[DOC]) is generally lower during spring runoff (≈5.0) than on the other sampling dates (5.5–6.5).

Ecotoxicological Ramifications

In the Quebec North Shore salmon rivers, hatching of fertilized salmon eggs generally occurs after snowmelt (G. Walsh, Department of Fisheries and Oceans, Institut Maurice Lamontagne, Ste-Flavie (Québec), pers. comm.). The salmon life stages that experience the spring pH and alkalinity depression are thus the eyed eggs and any juvenile salmon (1+, 2+) present in the river. Of these stages, the juvenile stage is the more sensitive (Henriksen et al. 1984), particularly if smoltification has begun. Indeed, in comparative studies, Atlantic salmon smolts proved to be markedly more sensitive to low pH and moderate Al concentrations than other salmonid species (Rosseland and Skogheim 1984).

In a field setting, the response of the juvenile salmon will depend on the duration of the episode, the Al concentrations attained, the forms present (Al speciation), and the prevailing physicochemical conditions (notably [H+] and [Ca^{2+}]). Results from published bioassay experiments, carried out in Norway on acidic and soft natural waters ([Ca] < 50 μM; see Table 3), suggest that concentrations of labile inorganic Al of about 2.6–3.3 μM (60–70 μg/L) may be acutely toxic over an exposure period of ≤6 d in low-Ca waters at moderately low pH (≈5.0). Under similar conditions, labile Al concentrations ≤1.5 μM are tolerated by juvenile salmon, suggesting quite a sharp toxicity threshold.

The physicochemical conditions prevailing during spring runoff in the salmon rivers we studied approach those found to be acutely toxic to salmon smolt in the Norwegian studies, but appear slightly less stressful (Table 3). For example, only in the Aux Rochers River were pH values less than 5.2 recorded, and Ca concentrations in all three rivers are consistently higher than in the Norwegian waters; in interpreting their field observations of salmon mortality during snowmelt, Henriksen et al. (1984) suggested that at levels above about 25 μM, Ca affords appreciable protection against the deleterious effects of Al. We conclude that springtime conditions in our Quebec North Shore rivers, although stressful, should not kill juvenile Atlantic salmon. This conclusion is consistent with the results of in situ bioassays carried out with juvenile presmolt salmon (age 2+) in the De la Trinité and Aux Rochers rivers during the 1988 spring runoff; although the test fish showed signs of stress (hematological parameters), no significant mortality was observed (A. Kemp, Department of Fisheries and Oceans, Institut Maurice Lamontagne, Ste-Flavie (Québec), pers. comm.).

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Unidirectional Natural Hybridization between Brown Trout (*Salmo trutta*) and Atlantic Salmon (*S. salar*) in Newfoundland

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McGowan, C., and W. S. Davidson. 1992. Unidirectional natural hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) in Newfoundland. Can. J. Fish. Aquat. Sci. 49: 1953–1958.

Protein electrophoresis and mitochondrial DNA analysis were used to detect the frequency and direction of natural hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) in nine Newfoundland rivers. In total, 37 hybrids were discovered in a sample of 792 juvenile fish for a regional frequency of 4.67%. Local frequencies ranged from 0.00 to 18.75% and were significantly heterogeneous. All of the hybrids sampled were produced from matings between female brown trout and male Atlantic salmon. Possible reasons for the breakdown of prereproductive isolating mechanisms between these species are considered. Reproductive characteristics of the populations involved appear to have a major influence on the dynamics of hybridization between these species in Newfoundland. It is proposed that an abundance of sexually mature Atlantic salmon parr in Newfoundland streams is responsible for both the frequency and direction of hybridization observed in this study.

L'électrophorèse des protéines et l'analyse de l'ADN mitochondrial ont été utilisées pour repérer la fréquence et l'orientation de l'hybridation naturelle entre la truite brune (*Salmo trutta*) et le saumon de l'Atlantique (*S. salar*) dans neuf cours d'eau de Terre-Neuve. Au total, on a découvert 37 hybrides dans un échantillon de 792 poissons juvéniles, soit une fréquence régionale de 4,67 %. À l'échelle locale, la fréquence allait de 0,00 à 18,75 %, et montrait une hétérogénéité significative. Tous les hybrides capturés provenaient de l'accouplement d'une truite brune femelle et d'un saumon de l'Atlantique mâle. Nous examinons les raisons possibles de la rupture des mécanismes d'isolement pré-reproduction entre ces espèces. Les caractéristiques de reproduction des populations étudiées semblent avoir une importance majeure dans la dynamique de l'hybridation entre ces espèces à Terre-Neuve. Nous posons que l'abondance de tacons de saumon de l'Atlantique ayant atteint la maturité sexuelle est responsable de la fréquence et de l'orientation de l'hybridation qui ont été observées au cours de notre étude.

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Naturally occurring hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) was first reported on the Avalon Peninsula of Newfoundland by Verspoor (1988). At the time, the observed hybrid frequency of 0.9% was relatively high when compared with values of 0.3–0.4% recorded in the United Kingdom (Payne et al. 1972; Solomon and Child 1978; Crozier 1984). This difference was attributed to the fact that brown trout are not indigenous to North America (MacCrimmon and Marshall 1968). Hubbs (1955) has suggested that hybridization is more likely to occur where species are introduced because prereproductive isolating mechanisms will not be as developed as they are between naturally sympatric species. The last introduction of brown trout in Newfoundland was made in 1906 (Andrews 1965). Since that time, brown trout have colonized many rivers on the Avalon Peninsula where they currently coexist with Atlantic salmon.

More recently, these species have been found to hybridize at frequencies of 2.3% in Spanish rivers (Garcia de Leaniz and Verspoor 1989) and 13% in Swedish rivers (Jansson et al. 1991) where both brown trout and Atlantic salmon are native. Such high frequencies suggest that specific ecological conditions may be responsible for the breakdown of prereproductive isolating mechanisms. In Newfoundland, other factors besides the introduction of brown trout may act to encourage natural hybridization between these species.

In addition to the introduction of nonindigenous species, Hubbs (1955) identified habitat degradation, limited spawning area, and disparity in species abundance as potential causes behind hybridization in fish. To these, Mayr (1970) has added that hybridization is more likely to occur if the mating bond is weak. These points are often cited as possible mechanisms behind natural hybridization, but there is rarely enough information available to establish which of these conditions, if any, is a predominant cause in a particular case.

More information regarding the dynamics of this interspecies interaction can be obtained through mitochondrial DNA (mtDNA) analysis of hybrid fish (Avise and Saunders 1984; Gyllensten et al. 1985a; Campton 1987; Herke et al. 1990). The mitochondrial genome is maternally inherited (Hutchinson et al. 1974; Gyllensten et al. 1985b), and mtDNA from both brown trout and Atlantic salmon has been characterized (Gyllensten and Wilson 1987; McVeigh et al. 1991). By identifying the genotype of mtDNA found in a hybrid fish, the maternal species, and thus the direction of hybridization, can be determined.

This study combines protein electrophoresis and mtDNA analysis to determine both the frequency and the direction of hybridization between brown trout and Atlantic salmon in Newfoundland. This information is used to identify possible reasons for the breakdown of reproductive isolating mechanisms between these species.

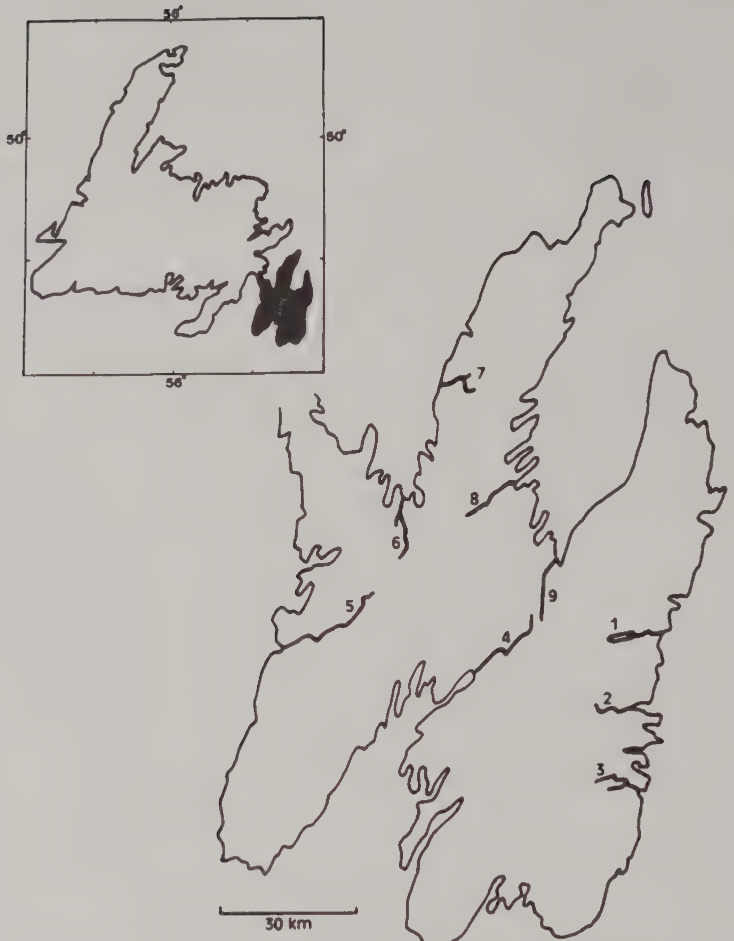

FIG. 1. Locations of sampled rivers. Numbers refer to river names listed in Table 1.

Materials and Methods

Samples of brown trout and Atlantic salmon parr were collected from nine river systems on the Avalon Peninsula of Newfoundland during a period from August 24, 1990, to November 30, 1990 (Fig. 1; Table 1). Some of the rivers were sampled at more than one site (Table 1). Every river system has been reported to have anadromous populations of both brown trout and Atlantic salmon (Porter et al. 1974; T. R. Porter and R. J. Gibson, Department of Fisheries and Oceans, St. John's Nfld., pers. comm.). No introductions of either brown trout or Atlantic salmon have been made into any of the rivers in this study. All salmon populations are natural and all brown trout populations have colonized the rivers by way of the ocean. Parr were caught by electrofishing, killed in the field using an overdose of anaesthetic, and then packed on ice and transported back to St. John's where they were stored at −70°C.

Hybrids were identified using allozyme electrophoresis. Skeletal muscle (0.2 g) was homogenized in an equal volume of 0.01 M Tris–HC1 (pH 7.5) and then centrifuged at 10 000 × g for 1 min. The supernatant was electrophoresed through an 11% horizontal starch gel at 30 $V \cdot cm^{-1}$ for 3 h using a Tris–citric acid/borate buffer system described by Ridgway et al. (1970). Gels were stained for the enzymes glucose-6-phosphate isomerase (EC 5.3.1.9; *Gpi-1,2,3*) and phosphoglucomutase (EC 5.4.2.2; *Pgm-1,2*) using standard techniques (Harris and Hopkinson 1976). These stains detect a total of five independent genetic loci, four of which are diagnostic for detecting hybrids of brown trout and Atlantic salmon (Vuorinen and Piironen 1984). This procedure has been used to identify natural hybrids in both Europe (Crozier 1984; Garcia de Leaniz and Verspoor 1989; Jansson et al. 1991) and North America (Beland et al. 1981; Verspoor 1988). Each species is fixed at four of the five loci for proteins with different electrophoretic mobilities. Hybrids express the sum of both parental types.

The maternal lineage of hybrid fish was identified by amplifying a segment of the mitochondrial cytochrome *b* gene using the polymerase chain reaction (PCR) (Saiki et al. 1988), followed by direct nucleotide sequencing. The preparation of DNA from skeletal muscle, amplification, and direct sequence analysis of the mitochondrial cytochrome *b* gene were performed using techniques described in detail by Bartlett and Davidson (1991). The cytochrome *b* gene was chosen, as it has already been characterized for both brown trout and Atlantic salmon by McVeigh et al. (1991). The nucleotide sequences of the cytochrome *b* genes of these species differ by a minimum of 5.2%.

TABLE 1. Sample sites and the number of brown trout, Atlantic salmon, and hybrids collected at each.

Sample site	N	Brown trout	Atlantic salmon	Hybrids[a] No.	Hybrids[a] %
1. Mobile R.					
a.	34	8	26	0	0.00
b.	77	31	46	0	0.00
Total	111	39	72	0	0.00
2. Cape Broyle R.	33	16	15	2	6.06
3. Renews R.	78	21	53	4	5.13
4. Salmonier R.	75	58	15	2	2.66
5. Northeast Placentia R.					
a.	34	25	8	1	2.94
b.	43	19	19	5	11.63
c.	32	14	18	0	0.00
Total	109	58	45	6	5.50
6. Spread Eagle R.	79	74	4	1	1.26
7. Hearts Delight R.	80	33	32	15	18.75
8. North R.	126	13	107	6	4.76
9. North Arm R.	101	51	49	1	0.99
Overall	792	362	393	37	4.67

[a] All hybrids had brown trout as a maternal parent.

The diagnostic segments used to identify the mtDNA of each species are shown in Fig. 2.

Results

Overall, 792 brown trout and Atlantic salmon were sampled, 37 of which were determined to be hybrids for a regional frequency of 4.67% (Table 1). Hybrids were found in all river systems except one, the Mobile River. Local frequencies of hybridization at the various sample sites ranged between 0.00 and 18.75% (Table 1) and were significantly heterogenous (2 × 9 contingency table: $G_{adj} = 38.03$, $P < 0.001$, df = 8). This suggests that the conditions responsible for hybridization are variable from one location to another. However, if the sample from the Hearts Delight River, where the hybrid frequency was particularly high, is excluded from the analysis, frequency differences are no longer significant (2 × 8 contingency table: $G_{adj} = 13.49$, $P < 0.10$, df = 8).

All hybrids expressed a complete summation of both parental genotypes at each of the four diagnostic loci. There was no evidence of introgression. Backcrossing is considered unlikely between these species due to disruptions of meiotic pairing in fertile F1 hybrids (Johnson and Wright 1986).

All of the hybrids had mitochondrial chytochrome *b* genes characteristic of brown trout. Therefore, all hybrids were the products of matings between female brown trout and male Atlantic salmon. Whether Atlantic salmon or brown trout was the predominant species in a river made no difference with respect to the direction of hybridization; the mothers of hybrid fish were always brown trout.

Discussion

The overall hybrid frequency of 4.67% observed in this study was much higher than the 0.9% reported by Verspoor (1988) for the same region. The lower estimate in the earlier survey is probably the result of sampling biases. Verspoor (1988) analyzed only those fish identified morphologically as Atlantic salmon and included samples from rivers where no brown trout had ever been reported. Morphologically, hybrids can resemble either species (Jones 1948; Nyman 1970), so surveys must include a random sample of brown trout and Atlantic salmon in order to obtain an accurate estimation of hybrid frequency. In addition, low hybrid viability may prevent many fish from reaching adult size. The present survey only considered samples of juveniles whereas Verspoor (1988) included parr, smolts, and adult fish. If only samples of parr are considered and those from rivers known not to contain brown trout are excluded, then the hybrid frequency in the survey by Verspoor (1988) becomes 2.2% which is more similar to the results presented above.

Crozier (1984) considered only brown trout and sampled both adult and juvenile fish in determining a hybrid frequency of 0.4% in the Lough Neagh system of Northern Ireland. The combined surveys of Payne et al. (1972) and Solomon and Child (1978) proposed a 0.3% hybrid frequency for the United Kingdom. However, these studies only considered adult Atlantic salmon caught offshore in commercial fishing nets.

It is probably more appropriate to compare results of the present study with those which have sampled only juvenile Atlantic salmon or brown trout. In North America, Beland et al. (1981) sampled juveniles of both species from the Stewiacke River, Nova Scotia, and found one hybrid out of 56 fish for a frequency of 1.8%. Hurrell and Price (1991) sampled both Atlantic salmon and brown trout parr when estimating a hybrid frequency of 1.4% for rivers in south west England. Garcia de Leaniz and Verspoor (1989) found that 2.3% of Atlantic salmon parr sampled in Spanish rivers were hybrids. Jansson et al. (1991) discovered a hybrid frequency of 13% in samples of brown trout and Atlantic salmon parr from the River Gronan, Sweden, with local frequencies as high as 23%. However, the last two cases may have higher than normal hybridization frequencies due to former introductions of Atlantic salmon in the rivers which were sampled.

It is difficult to say whether frequencies of hybridization are actually higher in North America, where brown trout are introduced, than they are in Europe, where both species are naturally sympatric. Hybridization between these species is possible in North America only because of the introduction of brown trout. What is not obvious is how prereproductive isolating mechanisms between these species have been compromised such that

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Salmo trutta: TTATATAGGTAGGAACCATAGTAGAGTCCTCGGGCGATATG
                      *           *  *              *
Salmo salar:  TTATATAGATAGGAACCATAATAAAGTCCTCGGGCGATGTG

Salmo trutta: TATATAAATACAGATAAAGAAGAAAGATGCTCCGTTAGCGT

Salmo salar:  TATATAAATACAGATAAAGAAGAAAGATGCTCCGTTAGCGT

Salmo trutta: GAATGTTTCGGATGAGTCAGCCGTAGCTAACATATCGGCAA
                     *              *
Salmo salar:  GAATGTTACGGATGAGTCAGCCATAGCTAACATATCGGCAA
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FIG. 2. Nucleotide sequences of segments of the cytochrome *b* genes of brown trout and Atlantic salmon. Asterisks indicate differences used to identify species.

extensive hybridization is occurring in both Europe and North America. mtDNA analysis provides additional information and permits a closer examination of possible explanations.

Hybridization between brown trout and Atlantic salmon in Newfoundland appears to be unidirectional, with Atlantic salmon males always fertilizing brown trout females. The simplest explanation for this observation would be that there is a difference in viability between the reciprocal crosses such that only hybrids with brown trout mothers are surviving long enough to be sampled. Such was the case for natural hybridization between northern pike (*Esox lucius*) and chain pickerel (*E. niger*) in which the maternal parent of all hybrid fish was a chain pickerel (Herke et al. 1990). Previous experiments in the laboratory had shown that hybrids produced using chain pickerel females are viable whereas the reciprocal cross is completely inviable (Buss and Miller 1967). Breeding experiments comparing the relative viability of brown trout, Atlantic salmon, and their reciprocal hybrids have had variable results. Some showed that hybrids produced with brown trout eggs have higher survival (Refstie and Gjedrem 1975) whereas others have had greater success with hybrids produced using the eggs from Atlantic salmon or Atlantic salmon grilse (Hofer 1909, cited in Refstie and Gjedrem 1975; Alm 1955; Piggins 1970). Results from such experiments probably depend on the particular strains of fish used in the cross as well as the quality of eggs and milt at the time of fertilization (Chevassus 1979). Breeding experiments carried out by the present authors using sea-run brown trout and Atlantic salmon grilse from the Northeast Placentia River (Fig. 1; Table 1) suggest that hybrids produced from Atlantic salmon eggs are greater in viability than the reciprocal cross (McGowan and Davidson 1992). If natural hybridization had occurred between brown trout males and Atlantic salmon females, it is likely that the F1 products of these matings would have been detected along with those of the reciprocal cross. Since no such hybrids were observed, we conclude that hybridization between these species in Newfoundland is unidirectional, with salmon males always fertilizing brown trout females.

Hubbs (1955), in an extensive review of natural hybridization among fish, identified the degradation of habitat or a shortage of suitable spawning habitat as environmental factors likely to encourage cross fertilization. There is no evidence of habitat degradation in any of the sample sites. However, it is possible that in some streams, spawning habitat is limited and both species must share common spawning areas. Similar to an introduction, both conditions bring spatially isolated species together into the same spawning habitat. In this situation, hybridization should occur at random, with hybrid frequencies reflecting the probability of a male from one species encountering a female from the other species. Also, males and females of each species should contribute equally to hybrid matings. Although the loss of spatial segregation may explain the occurrence of hybridization between these species, it does not explain the observed bias in direction. Additional factors, other than environmental conditions, must be influencing the occurrence and dynamics of hybridization between these species in nature. Something particular to the salmon and trout populations themselves is likely to be responsible.

Hubbs (1955) has also suggested that considerable disparity in species abundance may promote hybridization. Avise and Saunders (1984) found that natural hybridization between species of sunfish (genus *Lepomis*) occurred most often when one of the species involved was rare. They also discovered, through mtDNA analysis, a tendency for the rare species to provide the female in hybrid matings. There are no data available on the relative abundance of breeding Atlantic salmon and brown trout in Newfoundland streams. Egglishaw (1970) has shown that the recruitment of Atlantic salmon and brown trout occupying the same stream can be very different. Consequently, juvenile species ratios are not an accurate representation of relative species abundance for adult fish. However, large differences in juvenile species abundance suggest that sample sites in the Spread Eagle and Salmonier rivers are predominantly brown trout streams whereas sample sites in the Renews and North rivers are predominantly salmon streams (Table 1). If hybridization is frequency dependent, then the direction of hybridization would be expected to reverse when the predominant species changes. This is not observed, and thus it is unlikely that disparity in species abundance is a principle cause of hybridization.

In his review of hybridization among vertebrates, Mayr (1970) considered the nature of the mating bond as an important factor influencing the strength of prereproductive isolating mechanisms between species. Monogamous relationships, such as those found among several bird species, require strong mating bonds. Specific reproductive behaviors have evolved in order to avoid making costly mistakes when choosing a mate. Among many fish species, the relationship between mates is short-lived and mating bonds relatively weak. Reproductive behaviors may not be as discriminating and the cost of the occasional hybridization to an individual may not be as great.

The reproductive behavior in *Salmo* has been extensively documented (Jones and Ball 1954; Campbell 1977; Webb and Hawkins 1989). After migrating upstream, a female will excavate a form or redd in the gravel substrate into which eggs will be deposited as they are fertilized. Large males physically defend access to a female from smaller, less aggressive males. The relative strength or weakness of the mating bond is questionable. Jones and Ball (1954) reported strong aggressive defense of the female by males, especially in brown trout. However, differences in the life history characteristics of brown trout and Atlantic salmon may explain the unidirectional nature of hybridization between these species in Newfoundland.

A bias in direction would be expected if each species spawned at a different time. Webb and Hawkins (1989) have found that Atlantic salmon females will arrive at a spawning site ahead of males and leave soon after spawning. Males, on the other hand, remain in the stream for a considerable time after spawning, possibly looking for additional mates (Greeley 1932; Webb and Hawkins 1989). Consequently, males of an early spawning species may still be present when females of a later spawning species arrive. In such a case, the later species would be prone to contributing females in hybrid matings and the earlier species the males. In Norwegian rivers, where salmon and trout are naturally sympatric, temporal difference in spawning time is thought to be the major mechanism of prereproductive isolation, brown trout having a peak spawning time approximately 15 d earlier than salmon (Heggberget et al. 1988). No comparative data are available on the spawning times of anadromous brown trout and Atlantic salmon in Newfoundland, although both species have been observed spawning at the same time on the North Harbour and Collinett rivers (M. O'Connell, Department of Fisheries and Oceans, St. John's, Nfld., pers. comm.).

In Newfoundland rivers, anadromous Atlantic salmon are generally larger than brown trout. Brown trout may exceed 50 cm in length in many Newfoundland rivers and lakes (Wiseman 1973). However, Lear and Day (1977) found on the North Harbour River that 78% of the upstream migrating anadromous brown trout had fork lengths ranging between 15 and 30 cm, while Atlantic salmon were 50–60 cm long. O'Connell (1982) reported the mean length of spawning anadromous male brown trout for the North Harbour, Northeast Placentia, and Colinet rivers to be 31 cm. Fish this size would weigh under 0.5 kg. Angling statistics for these rivers suggest that Atlantic salmon grilse are normally over 1.5 kg (Porter et al. 1974). This difference in size would allow large anadromous male salmon to outcompete smaller anadromous male brown trout for their mates. Subdominant anadromous male salmon that cannot gain access to conspecific females may be mating with brown trout females instead. However, in Newfoundland rivers, female salmon are generally more abundant than males (O'Connell and Reddin 1983) and competition between anadromous males is likely to be limited.

An alternative life history strategy employed by male Atlantic salmon and brown trout is to mature sexually within freshwater as parr (Dalley et al. 1983; Jonsson 1985; Gibson and Cunjak 1986; Myers et al. 1986; L'Abée-Lund et al. 1990). These individuals compete with one another for superior positions close behind an anadromous female (and attendant anadromous male if there are any present) and are able to sneak in and fertilize some of the eggs as they are shed (Jones and King 1952; Campbell 1977; Myers and Hutchings 1987). Crozier (1984) noted that in Irish rivers where hybrid frequencies were highest, mature parr were also particularly abundant. Garcia de Leaniz and Verspoor (1989) reported exceptionally high early sexual maturation among salmon parr in Spanish rivers where hybrid frequencies averaged 2.3%, but reached as high as 7.7%.

These mature male parr may not be as selective as anadromous males and may indiscriminantly sneak matings with large females of either species. Competition among parr may force subdominant males to seek matings with congeneric females instead. Moreover, sexually mature parr would be present in the stream during the spawning period of both species, whether or not there is temporal segregation of spawning times of the anadromous fish.

There is a considerable amount of interpopulation variation with respect to the proportion of and age at which salmon and trout mature as parr (Myers et al. 1986; L'Abée-Lund et al. 1990). This variability is heritable as well as environmentally influenced such that faster growing fish tend to mature at an earlier age (Thorpe et al. 1983). Atlantic salmon males in Newfoundland tend to mature sexually as parr more often and at an earlier age than brown trout males. In the North Harbour, Colinet, and Northeast Placentia rivers, male brown trout do not begin to mature until they are 3+ years of age, and only at 4+ and older will over 50% of a given year-class be sexually mature (O'Connell 1982). In contrast, Atlantic salmon males of many Newfoundland rivers become sexually mature as early as 1+ yr of age (Myers et al. 1986). Gibson and Cunjak (1986) found in the North Arm and Salmonier rivers (Fig. 1; Table 1) a higher percentage of Atlantic salmon parr maturing sexually and at an earlier age than brown trout. They attribute this difference to competitive interactions between the two species which have led to slower growth in brown trout and consequently later maturation. O'Connell (1982) has reported brown trout growth to be slower in Newfoundland than in Europe.

The greater tendency for Atlantic salmon to mature sexually as parr at an earlier age than brown trout can explain the bias in direction of hybridization observed in this survey. Since the majority of sexually mature parr in the streams would be salmon, most if not all matings of this type that produce hybrids would be expected to involve Atlantic salmon males and brown trout females. We suggest that the abundance of sexually mature Atlantic salmon parr in Newfoundland streams is responsible for both the frequency and direction of hybridization observed in this study.

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Morphological Differentiation of Lake Sturgeon (*Acipenser fulvescens*) from the St. Lawrence River and Lac des Deux Montagnes (Quebec, Canada)

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Guénette, S., E. Rassart, and R. Fortin. 1992. Morphological differentiation of lake sturgeon (*Acipenser fulvescens*) from the St. Lawrence River and Lac des Deux Montagnes (Quebec, Canada). Can. J. Fish. Aquat. Sci. 49: 1959–1965.

We investigated the degree of morphological differentiation of lake sturgeon (*Acipenser fulvescens*) from the St. Lawrence River (Lacs Saint-Pierre and Saint-Louis and the river itself downstream from Lac Saint-Pierre) and from Lac des Deux Montagnes. Forty-one morphometric characters (traditional and truss network) and two meristic traits were used on 200 sturgeon (890–1010 mm total length) from five sites. The study of the meristic characters showed a difference of 1.5 gill rakers between Lac Saint-Louis sturgeon and those from Lac Saint-Pierre and Lac des Deux Montagnes although the range was identical in all groups. Principal components and discriminant analyses performed on morphometric characters showed that Lac des Deux Montagnes sturgeon differ markedly from the St. Lawrence River fish by their proportionally higher and longer head (correct reclassification 89%). The more discriminant descriptors show a difference ranging from 4 to 16 mm between Lac des Deux Montagnes fish and those from the St. Lawrence River. The multivariate analyses also revealed significant morphometric differences between fish in different sectors of the St. Lawrence River. However, the absolute differences are rather tenuous and are not adequate to designate separate stocks based solely on morphology.

Nous avons évalué le degré de différenciation morphologique existant entre les groupements d'Esturgeons jaunes (*Acipenser fulvescens*) du fleuve Saint-Laurent (lacs Saint-Louis et Saint-Pierre, fleuve en aval du lac Saint-Pierre) et du lac des Deux Montagnes. Nous avons étudié 41 mesures morphométriques traditionnelles et en réseau et deux caractères méristiques sur 200 spécimens provenant de cinq sites (890 à 1010 mm, longueur totale). L'analyse des caractères méristiques révèle une différence moyenne de 1,5 branchicténies entre les esturgeons du lac Saint-Louis et ceux des lacs Saint-Pierre et des Deux Montagnes, malgré des intervalles de distribution égaux. L'analyse en composantes principales et les analyses discriminantes effectuées avec les descripteurs morphométriques ont montré que les esturgeons du lac des Deux Montagnes se distinguent nettement de ceux du Saint-Laurent par leur tête, proportionnellement plus haute et plus longue (reclassification correcte: 89 %). Les descripteurs les plus discriminants affichent des différences de l'ordre de 4 à 16 mm entre ces deux groupes. Les analyses multivariées ont également révélé l'existence de différences significatives entre les esturgeons des différents secteurs du Saint-Laurent. Ces différences, en valeurs absolues, sont toutefois ténues et ne sont pas suffisantes pour déclarer des stocks distincts sur la base de la morphologie seulement.

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With annual catches of approximately 200 metric tons (landed value $500 000) and annual yields in excess of 2 kg/ha in the two main commercial fishing sectors, Lacs Saint-Louis and Saint-Pierre (Fig. 1), the commercial fishery of the St. Lawrence River lake sturgeon (*Acipenser fulvescens*) is one of the most important in America (Dumont et al. 1987a, 1987b). Although this fishery has been in operation for more than a century, we know very little of the stock composition of these fish. Lac des Deux Montagnes, the last enlargement of the Ottawa River before its confluence with the St. Lawrence River, is also occupied by sturgeon which were commercially exploited before 1950. During the winter of that year, a major anoxia resulted in the extinction of most of the species in this lake (Mongeau et al. 1982). Commercial fishing has been prohibited since and the population has been slowly recovering. Since sturgeon can move freely between Lacs des Deux Montagnes, Saint-Louis, and Saint-Pierre, we do not know whether St. Lawrence River sturgeon contributed to this recovery.

Based on a mark–recapture study and on biological statistics obtained from the commercial catch (age and length composition, growth, and mortality), Fortin et al. (1991) concluded that the highly sedentary nature of tagged sturgeon from lacs des Deux Montagnes, Saint-Louis, and Saint-Pierre, together with differences in production parameters, justified that sturgeon from each of these fluvial lakes, and those from the fluvial corridor downstream from Lac Saint-Pierre, be considered as distinct management units. However, they showed also that sturgeon tagged on spawning grounds located in the Des Prairies and l'Assomption rivers (Fig. 1) were recaptured all along the St. Lawrence River (from Lac Saint-Louis to Lac Saint-Pierre), but not in Lac des Deux Montagnes. In contrast, fish tagged in the Lac des Deux Montagnes have never been recaptured on spawning grounds used by sturgeon from the St. Lawrence River. We used morphometric and meristic characters to examine the hypothesis that the four sturgeon management units defined by Fortin et al. (1991) are morphologically different and thus should be considered as distinct phenotypic stocks as

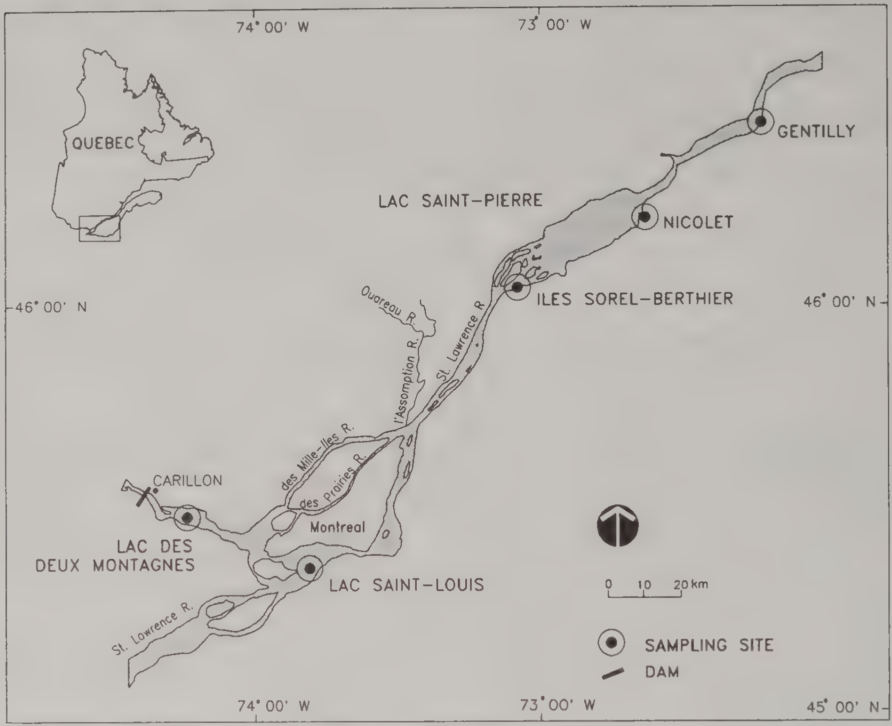
74° 00' W
73° 00' W
QUEBEC
GENTILLY
LAC SAINT-PIERRE
NICOLET
ILES SOREL-BERTHIER
46° 00' N
46° 00' N
Ouareau R.
St. Lawrence R.
l'Assomption R.
des Mille-Iles R.
des Prairies R.
CARILLON
Montreal
LAC DES
DEUX MONTAGNES
LAC SAINT-LOUIS
0 10 20 km
St. Lawrence R.
SAMPLING SITE
DAM
74° 00' W
73° 00' W
45° 00' N

FIG. 1. Localization of sampling sectors in the St. Lawrence River and of the rivers used as spawning grounds by the lake sturgeon in the Montreal Archipelago.

well. On a more fundamental basis, our study addresses the problem of the degree of morphological variation in sympatric groups of sturgeon.

Morphometrics and meristics are currently used to identify phenotypic stocks (Ihssen et al. 1981). Some authors used morphometrics to separate subspecies and populations of sturgeon (Vladykov 1955; Carlson et al. 1985; Artyukhin and Zarkhua 1986; Ruban and Sokolov 1987). For example, Ruban and Sokolov (1987) showed that water temperature and current velocity influenced the morphology of *Acipenser baeri* in the Lena River; they also reported morphological variation between sturgeon from the Lena and Aldan rivers, which might be related to differences in growth rate between the two rivers. We suggest that the relatively sedentary nature of sturgeon in Lacs des Deux Montagnes, Saint-Louis, and Saint-Pierre, together with environmental differences between these lakes, could induce some morphological differentiation between local groups of these fish.

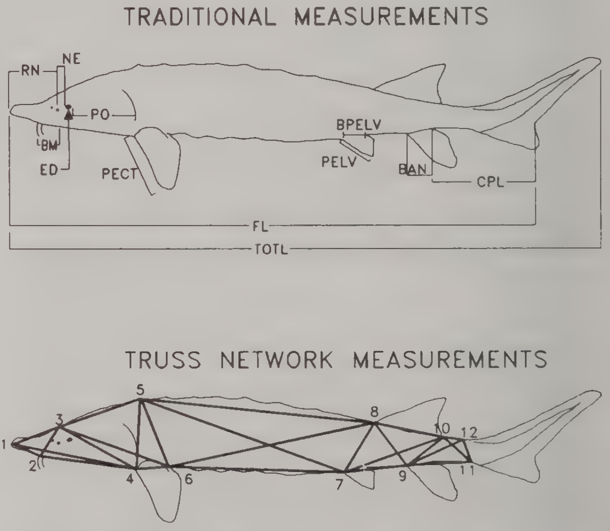
TRADITIONAL MEASUREMENTS
RN
NE
PO
BM
ED
PECT
BPELV
PELV
BAN
CPL
FL
TOTL
TRUSS NETWORK MEASUREMENTS
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 2. Illustration of traditional and truss network measurements used in this study. RN, rostrum–nostril; NE, nostril–eye; BM, barbels–mouth; PO, postorbital; ED, eye diameter; PECT, length of pectoral fin; PELV, length of pelvic fin; BPELV, length of the base of pelvic fin; BAN, length of the base of anal fin; CPL, caudal peduncle length.

Materials and Methods

Samples were obtained from the commercial fishery in Lac Saint-Louis, from the Îles Sorel-Berthier and Nicolet sectors of Lac Saint-Pierre, and from an experimental commercial fishery at Gentilly between June and August 1988 (Fig. 1). Using two sites from Lac Saint-Pierre (one ''island'' site and one ''lake'' site, Nicolet) reflects the geographic distribution of the commercial fishing effort in this lake and provides a check on within-lake variation of the variables studied. In lac des Deux Montagnes, fish were obtained through experimental fishing. In all cases, 19- to 20-cm stretched mesh gill nets were used. Forty fish were collected at each site. In order to minimize bias related to growth allometry, sturgeon measuring between 890 and 1010 mm total length were selected. At this length, sturgeon are immature and they show no growth difference between sexes (Fortin et al. 1991).

Morphometric traits were selected among both traditional and truss network measurements (Strauss and Bookstein 1982). Traditional measurements were selected from Vladykov (1955)

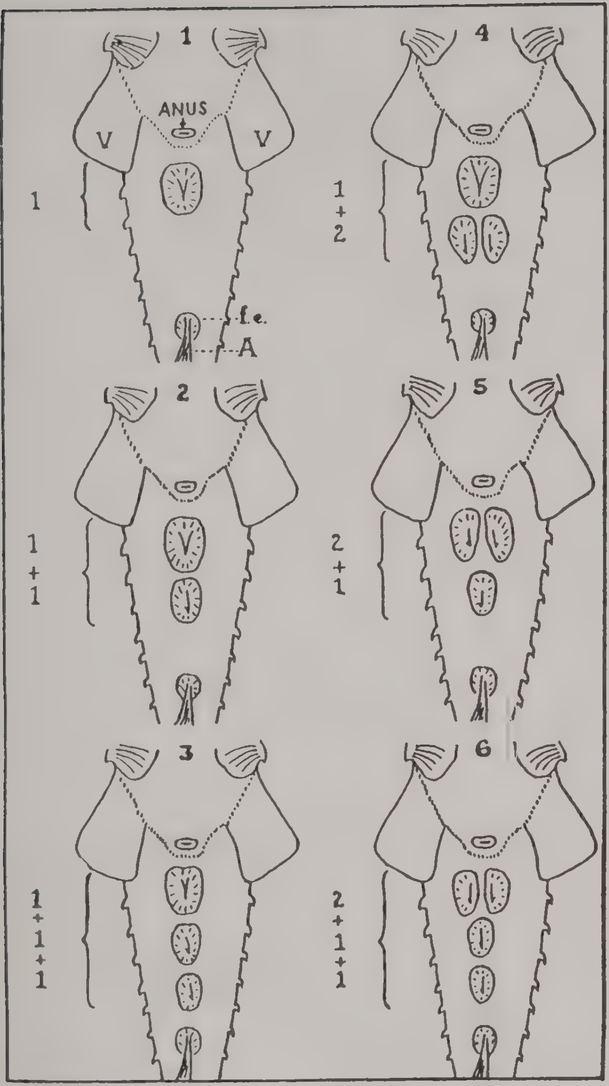

FIG. 3. Anatomical patterns of preanal plates (from Vladykov and Beaulieu 1946).

and Magnin (1963) who aimed to differentiate subspecies of *Acipenser oxyrhynchus* and from Roussow (1955) who attempted to differentiate two forms of *Acipenser fulvescens*. We selected interorbital width (IO), rostrum–nostril (RN), nostril–eye (NE), barbels–mouth (BM), and postorbital (PO) distances, eye diameter (ED), mouth width (MW), length of the pectoral (PECT) and pelvic (PELV) fins, length of the base of the pelvic (BPELV) and anal (BAN) fins and caudal peduncule length (CPL) (Fig. 2). Truss network meaurements were established using 12 landmarks, which serve to define five cells; the four sides and the two diagonals of each of these cells were measured (Fig. 2). All distances were measured on the left side of the fish. Total and fork lengths were measured to the nearest millimetre. One-metre-long homemade calipers, graduated in millimetres, were used for distances 5–7, 5–8, 6–7, and 6–8, while calipers graduated in 0.1-mm intervals were used for all other measurements.

Meristic characters included number and arrangement of preanal plates and number of gill rakers on the first branchial arch (Vladykov and Beaulieu 1946). The number and arrangement of preanal plates were tabulated according to the patterns described by Vladykov and Beaulieu (1946) (Fig. 3). The first left gill arch was cut off and gill rakers on the exterior and interior sides were counted in the laboratory.

Prior to the statistical analysis, the first 10 fish measured in Lac des Deux Montagnes were eliminated because of imprecise definition of the anatomical landmarks. The analyses were performed with 34 variables using a matrix of 134 fish from five sectors. Four descriptors (ED, MW, PELV, and IO) were discarded because they were difficult to measure accurately on sturgeon. Data greater or smaller than three standard deviations from the mean were considered as transcription or reading errors and recoded as missing values. Afterwards, all observations with missing values were discarded. Morphometric measurements were log-transformed to normalize their distribution. The various descriptors were correlated with fork length, but covariance analyses showed no slope differences between sectors. Following Claytor and MacCrimmon (1986), we used the total variance matrix, disregarding a priori groups, to standardize descriptors. Standardization was obtained using the formula

$$\mathrm{VARC} = \mathrm{VAR} - (\mathrm{M}\,(\mathrm{FL} - \mathrm{MFL}))$$

where VARC is the corrected variable, VAR is the original variable, M is the slope of the regression of the descriptor on fork length (FL), and MFL is the mean fork length of all fish. Principal component analysis (PCA) was used to explore the matrix variability, identify colinear variables, and help in choosing the right subset of variables to be used in the discriminant analysis, which verified the level of distinction between groups. Some morphometric characters were submitted to analysis of variance (ANOVA) and SNK multiple comparison tests. Univariate and discriminant analyses were performed using the SAS package (SAS Institute Inc. 1985); PCAs were performed using SPAD (Système Portable d'Analyse de Données) (Lebart et al. 1988a).

Results

In the PCA, Lac des Deux Montagnes sturgeon contributed the most to the formation of the two first axes, which explain 21 and 13% of the matrix variation, respectively. They can be distinguished from sturgeon from the St. Lawrence River, particularly those from Lac Saint-Louis and Îles Sorel-Berthier (Fig. 4). The centroids of these three groups are strongly correlated with the axes, meaning that they are distant by at least two standard deviations from the origin (Lebart et al. 1988b). The variables which are more strongly correlated with the axes are illustrated in the insets near the quadrant to which each group is related. Thus, Lac des Deux Montagnes sturgeon have a deeper and longer head (measurements 1–4, 2–4, 3–4, 1–3, 3–6, 2–3, and PO) than sturgeon from the St. Lawrence (Fig. 4). Sturgeon from Sorel differ by their longer body measurements (5–8, 5–7, 6–8, and 6–7) and sturgeon from Lac Saint-Louis by a longer and wider caudal peduncule (7–10, 9–10, 9–12, 9–11, 10–12, and CPL) and a longer anal fin base (BAN).

The performance of a particular subset of variables was evaluated on the basis of the percentage of correct reclassification in a discriminant analysis, the Mahalanobis distance between centroids and its associated probability, the value of Wilks lambda, and from the consistency of the variable selection by STEPWISE and BACKWARD procedures. The Kappa statistic was used to determine the improvement over chance of the clas-

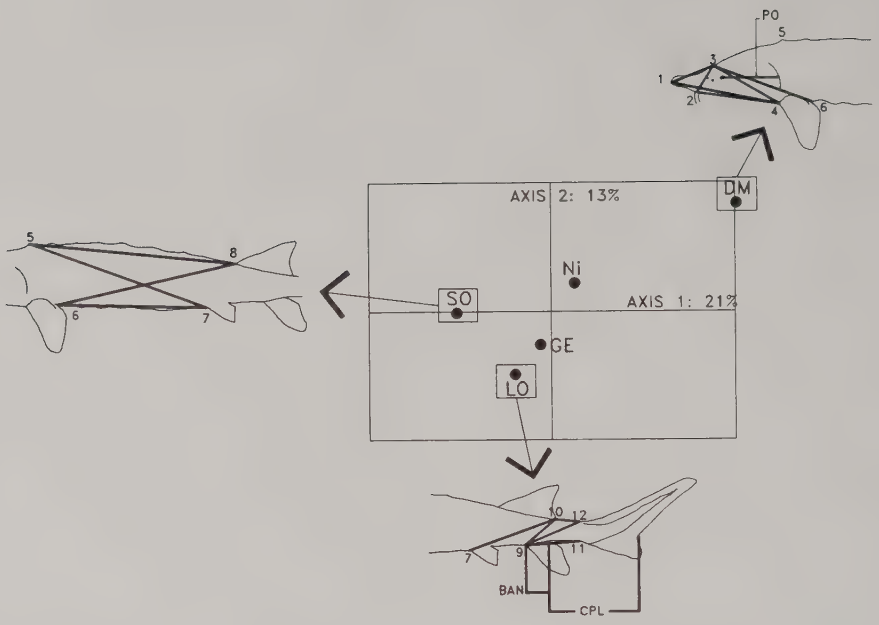

FIG. 4. Projection of the centroids of the five sectors on the first two principal components and illustration of the most important descriptors. Centroids shown in a square are significantly correlated with the axis. Percentages are the fraction of the variance explained by each axis. DM, Lac des Deux Montagnes; LO, Lac Saint-Louis; SO, Îles Sorel-Berthier; Ni, Nicolet; GE, Gentilly.

TABLE 1. Results of two discriminant analyses performed on sturgeon from the five sectors using four and eight descriptors. DM, Lac des Deux Montagnes; LO, Lac St-Louis; SO, Îles Sorel-Berthier; NI, Nicolet; GE, Gentilly. See Fig. 2 for an explanation of the abbreviations of the variables.

Variables	nb. var.	% reclassified Kappa		Distance between centroids					Wilks' lambda probability	Variable selection	
										Selected	Rejected
6–8, 9–11,	8	76%		DM	LO	SO	NI	GE	0.39	6–8	10–11,
10–11, 9–10,		0.70	DM	—	0.0001	0.0001	0.0005	0.0001	$p = 0.0001$	9–11,	9–10,
CPL			LO	2.81	—	0.02	0.009	0.06		CPL	PO,
2–3, 1–4, PO			SO	2.74	1.17	—	0.005	0.008			2–3
			NI	1.70	1.26	1.32	—	0.02			
			GE	2.64	1.07	1.28	1.19	—			
6–8, 9–11	4	53%	DM	DM	LO	SO	NI	GE	0.53	6–8,	—
2–3, PO		0.40	LO	—	0.0001	0.0001	0.0025	0.0001	$p = 0.0001$	9–11,	
			SO	2.26	—	0.005	0.002	0.027		2–3	
			NI	1.99	1.05	—	0.026	0.005		PO	
			GE	1.25	1.12	0.91	—	0.024			
				2.05	0.90	1.06	0.92	—			

sification derived from the discriminant analysis (Titus et al. 1984). All discriminant analyses performed with different subsets of variables allowed the global separation of all groups, including Nicolet and Îles Sorel-Berthier, although the pairwise distance between groups was occasionally not significant. With eight variables, the percentage of correct classification was 76% (89% for Lake des Deux Montagnes) (Table 1; Fig. 5) which is 70% greater than it would have been by chance alone. The variable selection is unstable resulting from colinearity between variables. The best subset of four variables finally retained (Table 1) provided a correct classification of 53% (40% improvement over chance) and allowed the separation of all groups ($p < 0.05$).

ANOVA tests performed on variables 1–4, 2–3, PO, 6–8, 9–11, CPL, and 10–11 showed that groups are globally different and that differences are mainly due to Lac des Deux Montagnes sturgeon (Table 2). Differences between means of the various descriptors range from 4 to 16 mm. Kolmogorov–Smirnov tests showed that differences between groups of sturgeon from the St. Lawrence River are not consistent from one descriptor to another. St. Lawrence River groups are found distinct mainly on the basis of their frequency distributions which share common boundaries.

The arrangement of preanal plates showed very little variation. Over 85% of sturgeon (Table 3) had only one preanal plate (pattern 1). Thus, no differences among groups could be dem-

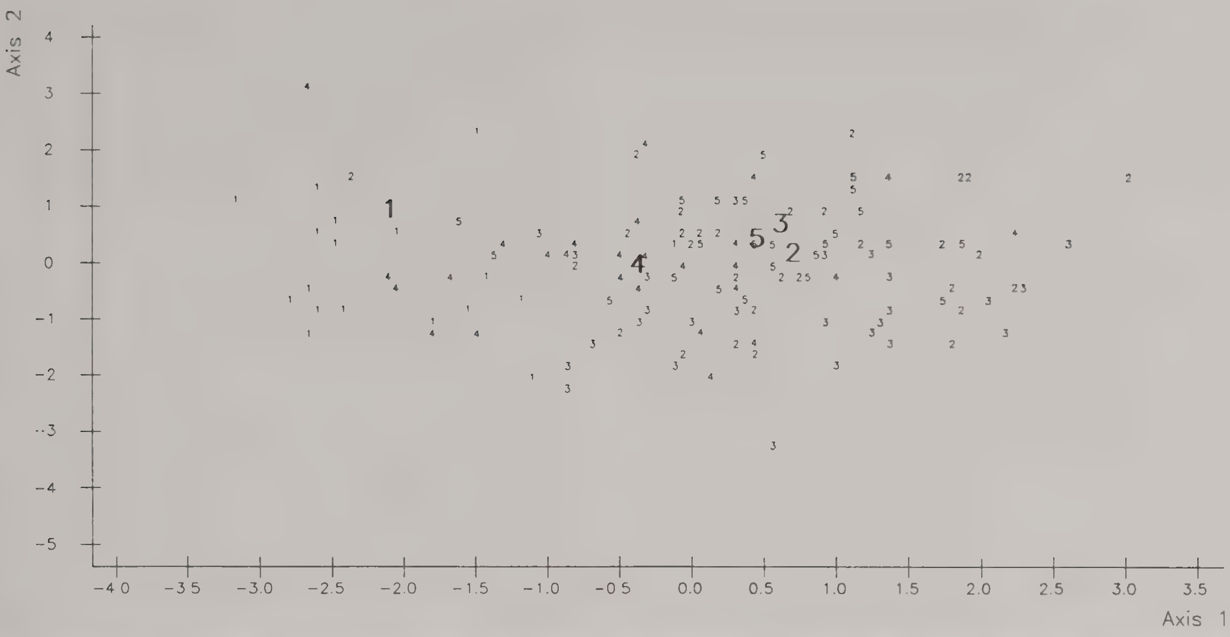

FIG. 5. Scatterplot of sturgeons from the five sectors in the canonical space generated by a discriminant analysis using eight descriptors (see Table 1). Numbers in bold are group centroids. 1, Lac des Deux Montagnes; 2, Lac Saint-Louis; 3, Îles Sorel-Berthier; 4, Nicolet; 5, Gentilly.

TABLE 2. Results of the ANOVA and of the Kolmogorov–Smirnov tests comparing the five sectors two by two. Variables are explained in Fig. 2.

	ANOVA			
Variable	Global model	Pairs of groups found different	Absolute difference between the means (mm)	Kolmogorov–Smirnov, pairs of groups found different
1–4	$p = 0.0001$	DM–LO	10.5	DM–LO
		DM–SO	11.7	DM–SO
		DM–NI	7.4	DM–NI
		DM–GE	8.1	DM–GE
				SO–NI
				SO–GE
2–3	$p = 0.04$	None		DM–SO
				LO–SO
				SO–GE
PO	$p = 0.0001$	DM–LO	4.4	DM–LO
		DM–SO	5.4	DM–SO
		DM–GE	6.3	DM–GE
6–8	$p = 0.0001$	DM–LO	14.1	DM–LO
		DM–SO	16.5	DM–SO
		DM–NI	9.5	DM–GE
		DM–GE	12.3	LO–NI
				SO–NI
9–11	$p = 0.0045$	DM–LO	5.7	DM–LO
		LO–NI	4.5	LO–SO
CPL	$p = 0.03$	DM–LO	5.8	DM–L0
10–11	$p = 0.0001$	DM–LO	6.4	DM–LO
		DM–SO	3.8	DM–SO
		DM–NI	3.7	DM–NI
		DM–GE	5.7	DM–GE
				LO–SO

TABLE 3. Disposition of preanal plates found in sturgeon of the five sectors. No specimens had patterns 4–6.

Pattern of the plates	Sector					
	DM	LO	SO	NI	GE	Total
1	35 85%	36 90%	34 89%	32 89%	33 89%	170 89%
2	5 12%	2 5%	4 11%	3 8%	4 11%	18 9%
3	1 2%	2 5%	0 0%	1 3%	0 0%	4 2%

TABLE 4. Comparison of the number of gill rakers using Kruskal–Wallis tests. Above the diagonal is the absolute difference between the means of groups which are significantly different (below the diagonal.) ns, not significant; $*p < 0.05$; $**p < 0.01$.

	DM	LO	SO	NI	GE
DM	—	1.1	ns	ns	ns
LO	*	—	1.9	1.4	ns
SO	ns	**	—	ns	ns
NI	ns	**	ns	—	ns
GE	ns	ns	ns	ns	—

onstrated. The range of number of gill rakers on the interior side of the branchial arch, 27–41, is the same for all the groups of sturgeon. However, Kruskal–Wallis tests showed significant global differences between groups ($p < 0.05$). The maximal divergence (1.9 rakers) occurred between sturgeon from Lac Saint-Louis and Îles Sorel-Berthier in Lac Saint-Pierre (Table 4). The numbers of gill rakers on the interior side of the first gill arch were similar to the range reported by Vladykov and Beaulieu (1951) (27–40). The number of gill rakers on the exterior side of the first gill showed no variation between groups.

Discussion

Lac des Deux Montagnes sturgeon were quite different morphologically from those of the St. Lawrence River. Such a level of distinction is consistent with their sedentary nature (Mongeau et al. 1982): approximately 95% of the sturgeon tagged in Lac des Deux Montagnes over a 15-yr period were recaptured in the same water body. It is also consistent with the absence of exchanges, on the spawning grounds, of sturgeon from Lac des Deux Montagnes and from the St. Lawrence River. Based on a mitochondrial DNA restriction fragment length analysis, Guénette (1991) has recently shown that small genetic differences exist between Lac des Deux Montagnes and St. Lawrence River sturgeon: both groups had one specimen showing a mtDNA genotype which was absent from the other group whereas all the other specimens had the same genotype. However, mtDNA genotype is not known to be related to morphological phenotype, and the morphological differentiation of Lac des Deux Montagnes sturgeon does not necessarily imply genetic divergence from St. Lawrence River fish. The slower growth rate of Lac des Deux Montagnes sturgeon (Mongeau et al. 1982; Fortin et al. 1991) could be responsible for the distinctive head proportions. Ruban (1989) reached the same conclusion when comparing groups of Siberian sturgeon (*Acipenser baeri*) from the Aldan and Lena rivers. Sturgeon from the Lena River grew more slowly and had a relatively larger head than those from the Aldan River.

Martin (1949) has shown that the relative sizes of the head and other body parts are affected by environmental conditions prevailing during the first stages of development. The logarithmic growth curve of an anatomical structure compared with the body length is characterized by inflexion points or slope variations which define growth stanzas. Environmental variation altering the growth rate could modify the length at which the fish reach the inflexion point and could induce variation in the body proportions. The relative length of the head of the lake sturgeon shows an inflexion point when the fish reach a standard length of approximately 34 mm (Harkness, cited in Martin 1949). The variation in the relative proportions of the head among sturgeon groups in the present study could thus be caused by environmental conditions prevailing during larval and juvenile development.

The number of plates and the number of rays in unpaired fins of sturgeons may be influenced by environmental conditions during the larval stage (Ruban and Sokolov 1987). In coregonines, gill-raker count has a high genetic component but can be modified by the presence of sympatric species, availability of prey, and growth rate (Lindsey 1981). We found no evidence of such influences on sturgeon gill rakers in the literature. However, the fact that morphometry or meristics can be influenced by conditions during development does not exclude genetic influences.

It is possible to separate the four groups of sturgeon from the St. Lawrence River (Lac Saint-Louis, Îles Sorel-Berthier, Nicolet, and Gentilly) with a linear combination of four morphometric descriptors. However, the observed differences for each of these variables, as well as for the number of gill rakers on the interior side of the first gill arch, are small and are not consistent in differentiating pairs of groups. Moreover, the linear combination of either four or eight descriptors might not be able to correctly classify new specimens because the morphological differences are so subtle between sturgeon from the various sectors of the St. Lawrence River. Finally, our results suggest that within-lake variations (Îles Sorel-Berthier versus Nicolet) are of the same order of magnitude as those observed between the more distant St. Lawrence River sectors. Thus, we conclude that St. Lawrence River sturgeon form a relatively homogeneous morphological group. Such an apparent contradiction between statistical and biological interpretation is not unique. Casselman et al. (1981) concluded that stocks of lake whitefish (*Coregonus clupeaformis*) in Lake Huron were not different enough to consider them distinct for management purposes, although multivariate analyses had proven them to be significantly different.

Our results do not confirm the hypothesis that the management units recognized by Fortin et al. (1991) in the St. Lawrence River are distinct phenotypic stocks. Moreover, the mtDNA analysis mentioned above showed that St. Lawrence River sturgeon form a homogeneous genotypic group (Guénette 1991). Thus, an integrative view of the stock composition of the St. Lawrence River sturgeon, and of the ensuing management implications, would be the following. These fish seem to form a homogeneous phenotypic and genotypic stock, which would seem to use common spawning grounds located in Des Prairies, Des Mille Îles, l'Assomption, and Ouareau rivers, all north shore tributaries of the St. Lawrence River (Fortin et al. 1991). Sturgeon larvae leave these spawning grounds and drift to the St. Lawrence River in June (LaHaye and Fortin 1990) and probably colonize the various sectors of the river as 0+ or older juveniles. The protection of these spawning grounds and of the juvenile habitat, yet to be characterized, becomes essential because their alteration could have negative effects on the stock as a whole.

Moreover, subadults and adults show a considerable degree of fidelity to a specified feeding area. Approximately 80% of fish tagged in Lacs Saint-Louis and Saint-Pierre (1981–86) were recaptured in their general area of tagging (Dumont et al. 1987b). Such behaviour, coupled with local environmental differences in the various sectors of the St. Lawrence River, is probably responsible for the differences in growth, length–weight relationship, and condition observed by Dumont et al. (1987b) and Fortin et al. (1991). Also, exploitation rates differ considerably between the various sectors of the St. Lawrence River, being highest in Lac Saint-Pierre, which explains local differences in total mortality rates and mean ages of the catch (Dumont et al. 1987b). We suggest that these groups of St. Lawrence River sturgeon, which differ on the basis of production parameters, should be managed on an individual basis in order to prevent local depletion in abundance and local deterioration of the commercial fishery. Further information on larval and juvenile ecology and on their dispersal from the spawning grounds would give a more complete image of the stock composition of Lac des Deux Montagnes and St. Lawrence River sturgeon.

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LETTERS AND COMMENTS/ LETTRES ET COMMENTAIRES

Mirror Image Stimulation, Social Hierarchies, and Population Differences in Agonistic Behaviour: A Reappraisal

Mirror image stimulation studies refer to experiments which measure the response of an animal that is confronted with its reflected image. In this paper I briefly describe the variety of questions on social behaviour and social structure in fish populations that have been addressed using a mirror image stimulation protocol. I first outline three significant advantages of using mirrors and then critically review some of the main limitations of the technique. I argue that, despite the advantages, mirror image stimulation experiments are not likely to provide further insight into questions regarding the evolution of social behaviour in fish populations, and thus, we need to look at alternative approaches. Failure to realize the limitations of this methodology can lead to misinterpretation, and thus, can have serious consequences for how we deal with important issues such as the interaction between populations of wild and hatchery origin.

Mirror image stimulation experiments with fishes have been used to address the relationship between agonistic behaviour and dominance (Baenninger 1970; Francis 1983, 1984), agonsitic behaviour and growth (Francis 1990), and agonistic behaviour, sex, and reproductive state (Holder et al. 1991). They have also been used to compare social behaviour among salmonid populations of wild origin (Taylor and Larkin 1986; Taylor 1988; Swain and Holtby 1989) as well as between populations of wild and hatchery origin (Swain and Riddell 1990). Lastly, they have been used in studies of competition for food resources (Metcalfe 1989) and cooperation during predator inspection behaviour (Milinski 1987, 1990; Dugatkin 1988).

Three advantages of using mirrors in studies of fish behaviour are commonly cited: (1) mirror experiments are operationally easy to standardize; (2) they remove the confounding effects of differential size and motivational state on dominance hierarchies (Metcalfe 1989; Swain and Riddell 1990); and (3) they provide highly reproducible aggression scores (Franck and Ribowski 1987; Francis 1990; Swain and Riddell 1990).

The major disadvantage is that the relationship between mirror-elicited aggression scores and agonistic behaviour in more natural situations is less than clear. The correlation between mirror scores and agonistic behaviour in a pairwise or group situation can be positive (Francis 1983, 1984; Metcalfe 1986), nonsignificant (Meliska et al. 1980; Franck et al. 1985; Franck and Ribowski 1987), or negative (Franck and Ribowski 1987). Mirrors provide a highly unnatural feedback compared with encounters between live opponents (Peeke and Peeke 1970; Rhoad et al. 1975; Reboreda and Kacelnik 1990; Ruzzante 1991). Gallup (1968) noted this characteristic of mirror stimulation, but instead of citing it as a disadvantage, he argued that it can be useful because it provides a continuous source of reinforcing, novel stimuli for the test individual. This may be an advantage when reinforcing stimuli are desired, but it imposes a major limitation of use of the technique. In a mirror test, a fish that performs a particular behaviour pattern is always responded to by a fish that does the same thing, and thus, it is impossible to know, for example, how a fleeing fish responds to an attacking fish, and vice versa.

In the context of the relationship between social hierarchies and mirror-elicited aggression scores, the most important limitation lies in the mechanism by which dominance relationships are established. The outcome of dominance encounters appears to be mostly determined by the order and relative frequency of different behaviours, such as lateral displays, chases, gill-cover erection, nips, bites, etc., in the eventual winners and losers (Simpson 1968; Frey and Miller 1972). Dominant individuals seem to have greater control over the course of the encounter, while subordinate fish appear to respond to the opponent's actions (Frey and Miller 1972). These subtle differences in sequence uncertainty or contingent probabilities cannot be detected during mirror image stimulation.

There is an additional, although related reason why an agonistic score from response to a mirror image correlates inconsistently with dominance status. The outcome of dominance encounters between pairs of individuals is likely to be determined not just by the individuals' baseline hormonal condition before exposure to stimuli, but also by the direction and intensity of hormonal changes during the course of a social interaction. These appear to differ both in magnitude and duration in the eventual winners and losers (Hannes 1985) (see also Greenberg et al. (1984) and Greenberg (1990) for similar results in lizards and Harding and Follett (1979) and Harding (1981) in birds). These changes determine the individuals' ongoing and/or future series of actions (Leshner 1975), a process referred to as a feedback effect on behaviour (Leshner 1979). Becuase of the nature of the stimuli, such changes cannot be detected, much less induced, during mirror image stimulation. It is not surprising, therefore, that serious attempts to correlate mirror aggression scores with dominance have often been unsuccessful (e.g. Francis 1983, 1984) and that attempts to correlate mirror aggression scores with size ranks have yielded results that are inconsistent across age- or size-classes (Francis 1990).

There is another limitation of mirror image stimulation experiments which is related to their use in assessing dominance relationships within populations. Social hierarchies as determined from any pairwise encounter, including mirror-elicited aggression scores, may not bear a direct relationship to the organized social structure of an interacting group of individuals (Baenninger 1968; Franck and Ribowski 1987). Staged pairwise contests between unfamilar individuals are usually followed by marked hormonal changes that differ in magnitude, duration, and direction between the eventual winners and losers (Hannes et al. 1984; Hannes 1985) (see also Bronson (1973) and Bronson et al. (1973) for similar results in mice and Sassenrath (1970) in monkeys). In contrast, differences in hormonal states between dominant and subordinate individuals in a stable social group are usually much less pronounced, if they exist at all (Hannes 1984) (see also Eaton and Resko (1974) for similar results in macaque monkeys and Ramenofsky (1984) in japanese quail). The fact that mirror-aggression scores in males of Siamese fightingfish (*Betta splendens*) do not correlate with dominance measured in round-robin trials but do correlate with dominance as expressed in a group situation (Baenninger 1968) indicates that the two dominance tests may be measuring different aspects of dominance behaviour. Braddock (1945) long ago recognized the existence of incomplete and nonlinear hierarchies in large social groups of fish. Newman (1956) recognized differences between what he called the ''definite'' social organization of a group of four individuals, in which dominance hierarchies were stable, and the ''indefinite'' social organization characteristic of a group of 20 interacting fish in which smaller individuals often returned nips to larger conspecifics. The probability that social hierarchies become nonlinear or ''nontransitive'' increases directly with group size (Chase 1982; Chase and Rohwer 1987). It is obvious, therefore, that dominance hierarchies determined from pairwise encounters such as mirror experiments may not provide an accurate description of the social organization or the overall intensity of agonistic behaviour of a large group of fish. Before extrapolating results from mirror image stimulation experiments to larger group situations, we need to know under what set of circumstances this extrapolation is valid.

In the context of comparing aggressive behaviour among wild fish populations or between wild and hatchery populations, there is little doubt that the between-population differences detected in the studies mentioned above (Taylor and Larkin 1986; Taylor 1988; Swain and Holtby 1989; Swain and Riddell 1990) are real. Fish from different populations or stocks of distinct origin react differently to a mirror image, even when raised from the egg in a common environment. However, the interpretation of these differences in terms of inherited aggressiveness remains difficult. One problem is that mirror experiments explicitly rule out density effects, and aggression may well be density related. This problem has implicitly been recognized in studies on chinook salmon (*Oncorhynchus tshawytscha*) (Taylor and Larkin 1986, Taylor 1988) and coho salmon (*O. kisutch*) (Swain and Holtby 1989) where, at least at the coarse population level, there appears to be a positive correlation between mirror-elicited behaviours and group tests for some but not all behaviours studied. Inferences concerning the magnitude and even the sign of differences in agonistic behaviour between populations in the wild based on mirror tests can be misleading.

The study of social behaviour and social structure in fish populations can be addressed by focusing on the mechanisms through which social hierarchies are established. Integrating mechanistic and functional approaches would facilitate our understanding of how social behaviour affects the dynamics of natural or cultivated fish populations. Some important advances in this direction have been made by Chase (1982, 1985, 1986) with the application of what has been defined as the jigsaw puzzle approach to the establishment of social hierarchies in small groups of fish and extended to larger group situations by Chase and Rohwer (1987). The method assumes that, if all successive pairwise interactions in a group are independent of previous interactions, then they all have an equal chance of occurring. It examines how successive pairwise interactions relate to each other and compares the observed sequence of interactions with all the different pairwise sequences that can possibly occur in such groups. The method can be useful in describing the behavioural processes involved in the development of hierarchies in fish populations and is appropriate for the study of dominance relationships as well as of agonistic interactions (Chase and Rohwer 1987).

In summary, population differences in the level of agonistic interactions estimated with mirror image stimulation can be difficult to interpret, partly because of the highly unnatural source of stimuli, but also because of the environment-dependent nature of behaviour. Studies attempting to correlate within-group dominance hierarchies and mirror-elicited aggression scores have often been unsuccessful. This is not surprising, since the outcome of dominance encounters between pairs of individuals appears to be determined mostly by the sequence and relative frequency of different behaviours in the eventual winners and losers, and these subtle differences in sequence uncertainty during a social interaction cannot be detected during mirror image stimulation. In addition, hormonal changes that occur during behavioural interaction between live opponents often differ in magnitude and/or direction in the eventaul winners and losers. These ongoing hormonal changes, in turn, affect the subsequent series of actions by a process referred to as a feedback effect on behaviour (Leshner 1979). This means that experiments attempting to measure dominance hierarchies or agonistic behaviour must allow each member of the pair or group to change its behaviour appropriately. Such experiments may seem ''messier'' because we have less control over the sequence of events, but such complexity seems to be a necessary part of a realistic behavioural investigation — Daniel E. Ruzzante, *Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1, Canada* (JB350)

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Through a Glass, Darkly: A Response to Ruzzante's Reappraisal of Mirror Image Stimulation Studies

In a reappraisal of mirror image stimulation (MIS) studies, Ruzzante (1992) first states that MIS experiments are unlikely to give ‘‘further insight into questions regarding the evolution of social behaviour in fish populations’’. Through his comment, he broadens his criticisms and finally concludes that MIS experiments have no merit for studies of social behaviour within populations or for comparisons of aggressive behaviour between populations. He implies that failure to realize the limitations of the MIS technique has led previous workers to misinterpret their results on agonistic behaviour in fishes. Ruzzante's arguments are flawed by logical inconsistencies and unreasoned inductive leaps, and he provides no evidence supporting his assertion that false conclusions have arisen through misinterpretation of MIS tests.

Most of Ruzzante's comments regarding the validity of MIS tests centre on their inability to accurately predict the hierarchical order of individuals within groups of fish. To illustrate this generalization, he discusses a study of Baenninger (1968). Ruzzante notes that MIS scores of male Siamese fightingfish (*Betta splendens*) do not correlate with dominance measured in round-robin trials but do correlate with dominance in a group situation. Contrary to Ruzzante, we conclude from these facts that dominance in a group of male Siamese fightingfish can be predicted from MIS tests (although not from paired contests). Having cited an example where MIS tests did provide insight into social organization, Ruzzante then claims it obvious that MIS tests may not provide such insights.

Ruzzante is correct in claiming that MIS may not predict position in social hierarchies. But this does not imply that MIS experiments have no merit for studies of social behaviour within populations. The most important limitation of MIS procedures in the study of social hierarchies is that aggressiveness itself appears to be unrelated to dominance ability in some vertebrates (Francis 1990), although not in others (Baenninger 1968; Rosenau and McPhail 1987). Thus, MIS techniques, which are generally used to measure aggressiveness in a controlled, repeatable fashion, do not reliably predict dominance in some species because what MIS tests measure, i.e. aggressiveness, does not predict dominance in those species. Other measures of aggressiveness that do not use mirrors are similarly unsuccessful at predicting dominance ability (Francis 1983, 1984). Francis, whose work Ruzzante repeatedly cites as showing the failure of MIS techniques (Francis 1983, 1984, 1990), and someone who, presumably, should have forsaken such a flawed technique, continues to publish work in which he and his coworkers use MIS techniques to measure aggressiveness and explore the role of aggressiveness in behavioural ecology of fishes (e.g. Holder et al. 1991). The discovery that aggressiveness and dominance are not necessarily intimately related is an important one, and one to which MIS tests were integral.

In discussing the physiological processes underlying the creation and stabilization of social hierarchies, Ruzzante argues that MIS scores cannot be expected to be correlated with dominance status because the hormonal differences observed between winners and losers of paired encounters cannot be induced or detected by MIS tests. But then he reverses his argument, asserting that MIS scores may be unrepresentative of dominance status in a group because the hormonal differences between winners and losers of paired encounters are not observed between dominant and subordinate individuals in a stable social group. If the hormonal differences between winners and losers of staged contests do not reflect dominance status in a group, as implied by Ruzzante, then the inability of MIS tests to detect such changes cannot be used as an argument against the validity of MIS tests for predicting dominance status. Aside from this logical circularity, we do not think it at all obvious that hormonal changes cannot be induced during MIS. We are not aware of any attempt to measure hormone levels during MIS testing but can see no reason why hormone levels would not change; the behaviours that coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) show in MIS tests are similar to behaviours seen in group encounters or "natural" situations (Taylor and Larkin 1986; Rosenau and McPhail 1987; Swain and Holtby 1989).

Having argued that MIS tests do not predict dominance ability, Ruzzante claims that it is also obvious that MIS tests may not provide accurate estimates of the levels of agonistic behaviours within large groups of fish. The social hierarchy within a group and the overall intensity of agonistic behaviour in the group are two quite different issues, and Ruzzante presents no evidence to substantiate his claim on this second issue. In fact, the evidence from between-population comparisons in coho and chinook salmon is that MIS tests do provide information on the relative intensity of agonistic interaction in groups of fish (Taylor and Larkin 1986; Rosenau and McPhail 1987; Taylor 1988; Swain and Holtby 1989).

Ruzzante's most valid point is that MIS tests cannot be used to study the mechanisms by which dominance relationships are established because they do not permit the full range of behavioural interactions between individuals. We fully agree with this well-established point (Landau 1951; Chase 1974, 1986). However, MIS tests have never been used for such studies in the fisheries and aquatic sciences literature (i.e. Rosenau and McPhail 1987; Taylor 1988; Swain and Holtby 1989; Swain and Riddell 1990). Instead, MIS techniques have been used to test for differences in agonistic behaviour between populations of coho and chinook salmon. We believe that MIS tests have been adequately validated for this purpose in these species. In both species, differences between populations in the levels of aggressive behaviours are in the same direction in MIS tests, paired contests, and group tests (Taylor and Larkin 1986; Rosenau and McPhail 1987; Taylor 1988; Swain and Holtby 1989).

Ruzzante criticizes previous interpretations of between-population differences in MIS behaviour because "aggression may well be density related". There is no evidence that the direction of between-population differences in aggressive behaviour depends on density in salmonids, so even that weak speculation is unjustified at this time. At any rate, the between-population correlation between MIS behaviour and behaviour among individuals appears to apply over a range of densities in coho and chinook salmon, i.e. in both paired and group (8–10 individuals) tests. We assert that Ruzzante's conclusion that inferences concerning between-population differences based on MIS are misleading is not only unsubstantiated but flies in the face of all published evidence.

We conclude that MIS tests provide useful information on at least the direction of between-population differences in aggressive behaviour in coho and chinook salmon. The technique is a useful one when it is not practical to perform the more difficult group tests with sufficient replication to draw statistical conclusions. Recent evidence indicates that some MIS behaviours are also correlated with dominance in group tests *within* populations of coho salmon (Holtby, et al. 1993). Thus, MIS scores may also be useful in some within-population applications such as selective breeding programs and quantitative genetic experiments (Riddell and Swain 1991) — L. Blair Holtby, *Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada*, and Douglas P. Swain, *Department of Fisheries and Oceans, Gulf Fisheries Centre, P.O. Box 5030, Moncton, N.B. E1C 9B6, Canada.* (JB478)

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